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BASAL METABOLISM IN OFFICE PRACTICE*

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THE facts which form the foundation for the basal metabolism test are not and have not been seriously questioned for a number of years. Some of the clinicians who have been slow to make use of this procedure have, we believe, not realized that any scepticism as to its value must be based on their doubt of the accuracy of the various recently marketed types of apparatus, and on the doubtful skill of the host of more or less trained technicians who are now making these tests. The value of the test depends primarily upon these factors. If we concede that these possible sources of unreliable readings are in the main controlled by the finding of normal readings in a series of normal individuals, and by close check on the abnormal readings, then the clinical usefulness may be said to depend upon whether or not the diagnostic assistance obtained is commensurate with the expenditure of time and energy necessary.

It is our intention to review the results which we have obtained in the last three years in the course of performing a total of six hundred tests on four hundred and fifty selected patients. We believe this review to be of some value for several reasons. The very simple technic which was used was not varied and the apparatus which was used was not changed during the entire time. (Several tests made with the Sanborn Handy Apparatus are not included in this report.) A large number of normal controls were tested. Many of the thyrotoxic patients were treated by x-ray and their tests were frequently repeated. Many of the patients showing a low metabolic rate were treated medically and their tests were repeated during treatment with thyroid substance or otherwise. No tests were performed in hospitals or at

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patients' homes. All patients came to the office on the morning the tests were made; consequently these results seem to be an index of the applicability of the method to office procedure.

The Benedict Portable Apparatus, manufactured by the Sanborn Company, was used. Patients were allowed no food for fourteen hours before arrival at the office. Long trips by street car or walking were discouraged and not allowed (when they could be avoided). After arrival, each patient was allowed to rest, clothed in a loose gown, in a comfortable bed. The room used was a comparatively quiet one. They were left undisturbed for one hour, or a longer period for rest was allowed whenever there was any indication of nervousness and particularly when the pulse rate seemed unsettled. A check for leaks was made and the soda lime was carefully watched to see that it was readily absorbing carbon dioxide. A preliminary try out of the apparatus with each patient, for a few minutes, seemed indispensable. A poorly arranged apparatus making the patient uncomfortable, will increase the oxygen consumption very materially. Ten minute periods were used. Where patients evidently were fatigued by a long period, shorter periods were used. Invariably a second period was tried and unless the readings of the several periods were within fairly close agreement, other tests were made. A temperature above 98.8° was regarded as abnormal and no dependence was placed upon the metabolic findings in such instances. All calculations were made on the basis of body surface area measurements calculated from the height-weight chart of DuBois.[1] Corrections for temperature and barometer were made and a respiratory quotient of 0.82 was assumed. The results were compared with the normal for the same age and sex.[2]

Tests have been performed on patients from eleven to seventy-seven years of age. We have found very few patients, in four hundred and fifty, from whom we could not get satisfactory cooperation.

Of the patients tested, the original test showed that the basal metabolic rate of two hundred and ninety-one (65– per cent) was between +10 and –10. The rate of one hundred and ten (24+ per cent) was above +10 and the rate of forty-nine (11– per cent) was below –10. In other words, one hundred and fifty-nine (35+ per cent) of four hundred and fifty patients were found to have abnormal basal metabolic rates, supposing the normal zone to be demarcated by the purely arbitrary figures +10 and –10. The forty-nine results obtained below –10 may be grouped as follows:

–10 to –15	17
–15 to –20	25
–20 to –40	7

Six of the seven between –20 and –40 were typical examples of myxedema. The seventh was a case of dementia precox who had taken very little food for several weeks.

Twenty-five of those below –10 fall in the group –15 to –20. Analyses of these cases show that six were diagnosed hypothyroidism four of whom improved, clinically, with appropriate treatment; one did not improve and

one refused treatment. Seven were diagnosed hypopituitarism. In each of these cases the clinical and x-ray evidence was sufficient for this diagnosis to be made without any consideration of the basal metabolic rate.

One patient had a basal metabolic rate of –17; one year after removal of an abscessed tooth, the basal metabolic rate was normal. Two had cyst adenomas of the thyroid of large size, with evident pressure symptoms. One of these cases is of particular interest: on tests eleven months apart, basal metabolic rates of –15 and –18 were obtained; one month after removal of the tumor, a normal basal metabolic rate was found. Another patient had a large colloid goiter; her condition was improved by treatment with thyroid substance.

Two patients, aged eleven years and eleven and a half years, were very much overweight for their height and age and seemed somewhat retarded mentally. As the standards for basal metabolism at this age are in dispute, no definite statement can be made in regard to these children. Clinically, they were thought to be cases of dyspituitarism.

Two patients had tuberculosis and were on a reduced food intake because of anorexia. Two patients were adults who clinically appeared to be merely obese. Against this being true, we have the records of many obese patients with normal basal metabolic rates and Means has found that the obese have normal basal metabolic rates.[3] One patient had dementia precox. The remaining one had had a double ovariectomy—no other evidence of glandular disturbance was found.

Of the seventeen in the group –10 to –15, seven, clinically, were thought to have mild hypothyroidism: five were distinctly improved with the use of thyroid substance; one was not improved and of one we have an incomplete record. Three were classed as cases of dyspituitarism. Two had adenomas of the thyroid. One had a goiter of adolescence. Two women had had double ovariectomies. On two patients no diagnosis was made; the most probable diagnosis on each was pulmonary tuberculosis.

In summary, it may be stated that of the forty-nine, there was definite evidence of some disturbance of the glandular system in thirty-nine, and it was thought to be highly probable in four more. Of the remaining six, four were poorly nourished individuals—two of the four probably had tuberculosis. According to McCann and Barr,[4] the loss in weight of patients with tuberculosis may be accompanied by a reduction in metabolism, which more than compensates for the tendency to increase caused by the disease. Finally, two had dementia precox in which condition low basal metabolic rates are not infrequent.[5]

On the basis of these figures, we believe that a basal metabolic rate below –10 is a significant finding and is, in the majority of instances, of some value in diagnosis.

Repetition of tests in cases of myxedema is valuable as an aid in deciding the dose of thyroid substance necessary to restore a normal basal metabolic rate.

Of the patients between +10 and −10, one hundred and forty-four were on the minus side and one hundred and forty-seven were on the plus side. We have gone over the records of these patients and have found twenty-one, formerly having hyperthyroidism, who have been treated by x-ray or surgically and who are now normal. In ten patients there was some evidence of dyspituitarism.

In addition to many cases of adolescent goiter and a few cases of probable colloid goiter, there were twelve patients with adenoma of the thyroid none of whom showed any evidence of thyrotoxicosis. We feel, as Boothby[6] does, that a normal basal metabolic rate eliminates hyperthyroidism as a diagnosis, and we believe that it is here that this test has its greatest field of usefulness. It is a self-evident fact that patients with so-called effort syndrome and many others with equally poorly understood symptom complexes, simulating toxic goiter, should not have their thyroid operated or x-rayed. We are depending more and more upon the basal metabolism test to eliminate these patients from the hyperthyroid group. It is certain that, with the history and physical findings alone, this is often a difficult thing to do.

We are not convinced that a normal basal metabolic rate is proof that the thyroid secretion is present in sufficient quantity, and we have seen several nontoxic goiters decrease materially in size and the condition of the patients improved considerably with the use of thyroid substance where the patients had normal basal metabolic rates.

We are also convinced that estimations of the basal metabolic rate, with our present knowledge of the functions of the pituitary, do little more than emphasize our lack of definite knowledge.

The one hundred and ten patients who had, on some occasion, a basal metabolic rate of above +10, may be grouped as follows:

+50 or above	19
+25 to +50	22
+15 to +25	17
+10 to +15	27

Of the nineteen above +50, sixteen had Graves' disease and three had toxic adenoma. Of the twenty-two between +25 and +50, seven had Graves' disease and thirteen had toxic adenoma. One was a decompensated cardiac who had a normal basal metabolic rate when compensation was restored by rest and digitalis. One patient had pernicious anemia.[7] Of the seventeen between +15 and +25, six had Graves' disease and five had toxic adenoma. An increase in body temperature from infection presumably accounted for the increased metabolic rate in two. Two were patients with moderately decompensated heart lesions. One patient had had food a few hours before the test. One patient had had no diagnosis made—the rate on three occasions had averaged +18.

In summary, the patients with basal metabolic rates from +15 up may be grouped as follows:

Graves' disease	29
Toxic adenoma	21
Decompensated cardiac	3
Pernicious anemia	1
Fever	2
Recent food	1
No explanation	1

In fifty of these fifty-eight patients, the basal metabolic rate was of considerable help. Primarily, it added to our knowledge of the severity of the disease in the individual patient. Secondarily, it gave a very good idea of the degree of success or of the failure of treatment and when the roentgen ray was being used, it was of help in deciding when it was no longer safe to give further treatment.

It is a significant fact that the only case of myxedema that we have seen, following the use of the roentgen ray in the treatment of toxic goiter, did not have the treatment checked up by basal metabolic rate estimations. We would feel less handicapped in caring for a patient with typhoid fever, without a thermometer, than we would in directing the treatment of a toxic goiter without basal metabolic rate estimations.

The group of patients whose tests read between +10 and +15 are of some interest. We believe that a rate between these limits should receive no consideration unless it is found elevated on several different days. These rates usually are not the basal rates. In other words, the majority of these rates are elevated because of fever, nervousness, movement or other factors and the true basal rate is probably within normal limits. This group of twenty-seven may be classified as follows:

Treated Graves' disease	3
Treated toxic adenoma	1
Hypertension and toxic (?) adenoma	1
Recent food	5
Moving	5
Fever	2
Uncertain cause	10

We have then come to consider results which fall between +15 and +10 as having no pathologic significance unless found in patients during the course of treatment for thyrotoxicosis.

CONCLUSIONS

Basal metabolic rate determination can be satisfactorily made in the physician's office.

The test is of value particularly: (1) In eliminating patients with pseudotoxic goiter from the group of patients with true thyrotoxicosis; (2) In classifying patients with thyrotoxicosis; (3) In following the treatment of these patients; (4) In the diagnosis of myxedema of varying grades of severity; (5) In giving a rational method for deciding the dose of thyroid substance necessary in the individual case of myxedema.

REFERENCES

[1]DuBois, D. and DuBois, E. F.: Formula to Estimate Approximate Surface Area, Arch. Int. Med., 1916, xvii, 863.
[2]Aub, J. C. and DuBois, E. F.: Arch. Int. Med., 1917, xix, 823.
[3]Means, J. H.: Studies of Basal Metabolism in Obesity and Pituitary Disease, Jour. Med. Research, 1915, xxxii, 121.
[4]McCann, W. S. and Barr, D. P.: Clinical Calorimetry, Arch. Int. Med., 1920, xxvi, 663.
[5]Bowman, K. M. and Grabfield, G. P.: Basal Metabolism in Mental Disease. Arch. Neurol. and Psychiat., March, 1923, ix, No. 3.
[6]Boothby, Walter M.: Endocrinology, January, 1921, v, No. 1.
[7]Meyer, A. L. and DuBois, E. F.: Basal Metabolism in Pernicious Anemia. Arch. Int. Med., 1916, xvii, 965.

THE EFFECT OF INSULIN UPON THE COMPLEMENT DERIVED FROM INSULINIZED GUINEA PIGS FOR THE WASSERMANN REACTION*

By Otto S. Kretschmer, A.M., M.D., Pueblo, Colo.

THE purpose of the following experimental work was to determine whether the hypoglycemia produced by the injection of insulin, or some other change produced in the blood by insulin might alter the action of complement derived from the guinea pig in performing the Wassermann reaction.

For this purpose four guinea pigs each weighing close to 250 grams were injected subcutaneously in the abdominal region each with five units of insulin. In about three hours another five units were administered. At varying intervals after these injections every one of the animals developed symptoms due apparently to the hypoglycemia, consisting of restlessness, scratching, crying followed by somnolence. Finally the animals lay on the side, being roused fairly readily by being touched. As soon as these symptoms had developed three of the animals were stunned by a blow on the back of the neck and bled from the external jugular vein. The blood was left to clot in the ice box overnight and some of the pooled serum was diluted 1:20 in the usual manner after the addition thereto of 0.425 gm. of NaCl per 10 c.c. of serum. The remaining animal was bled into a small quantity of potassium oxalate, and the blood sugar determined according to the method of Folin and Wu. This blood sugar was found to be only 11.9 mg. per 100 c.c. of blood.

All pigs were autopsied. No macroscopic pathology was found anywhere. The liver tissue was sectioned and stained according to Best's carmine method for the presence of glycogen. Only an occasional granule of glycogen could be found.

After the usual titration of the complement two known positive sera and two known negative sera were run for the Wassermann reaction in the usual manner, using for complement in one set of tubes the pooled serum of the guinea pigs treated with insulin, and in the other set using pooled serum

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derived from three normal untreated pigs. Two antigens were employed—the alcoholic extract of beef heart and Kolmer's new lecithinized-cholesterinized antigen obtained from the originator.

When reading the results of these Wassermann reactions no difference whatsoever in the action of the two kinds of complementary sera could be noticed —the two positive sera and the two negative sera being respectively positive and negative as had previously been found.

In order to ascertain the comparative strength of this complement derived from insulinized guinea pigs a "set-up" of ten tubes was arranged containing increasing quantities of this serum, and another set of ten tubes containing identical quantities of normal guinea pig complement was likewise arranged for comparison as shown in Table I.

TABLE I

TUBE	COMPLEMENT DILUTED 1:20 FROM NORMAL AND FROM INSULINIZED PIGS	ANTI-SHEEP AMBOCEPTOR	SHEEP CORPUSCLES 2.5 %	SALT SOLUTION 0.85 %	HEMOLYSIS PRODUCED BY SERUM		HEMOLYSIS PRODUCED BY SAME SERUM FROM INSULINIZED PIGS AFTER	
					FROM NORMAL PIGS	FROM INSULINIZED PIGS	20 DAYS	1 MONTH
1	.05 c.c.	2 Units	1.0 c.c.	1.25 c.c.	5%	None	None	None
2	.1	"	"	1.2	10	10%	5%	5%
3	.15	"	"	1.15	30	25	20	15
4	.2	"	"	1.1	60	50	70	40
5	.25	"	"	1.05	80	80	80	60
6	.3	"	"	1.0	100	100	90	80
7	.35	"	"	0.95	100	100	100	90
8	.4	"	"	0.9	100	100	100	100
9	.45	"	"	0.85	100	100	100	100
10	.5	"	"	0.8	100	100	100	100

It will be seen from the table that the complementary action of the serum derived from the insulinized pigs closely parallels that of the serum from normal ones, moreover the "insulinized" complement manifested excellent keeping properties even after one month's time.

CONCLUSIONS

The pooled serum of guinea pigs treated with insulin over a short period of time, in whom acute hypoglycemic symptoms have thus been created shows no appreciable change in its complementary titer in comparison with pigs not so treated, and retains this property for fully thirty days.

SENILE ATROPHY OF THE LIVER*

By Isolde T. Zeckwer, M.D., Boston, Mass.

IN the performance of postmortem examinations on an autopsy service composed largely of individuals past middle age, it becomes apparent that the extent to which atrophy of the viscera may occur as a senile change is

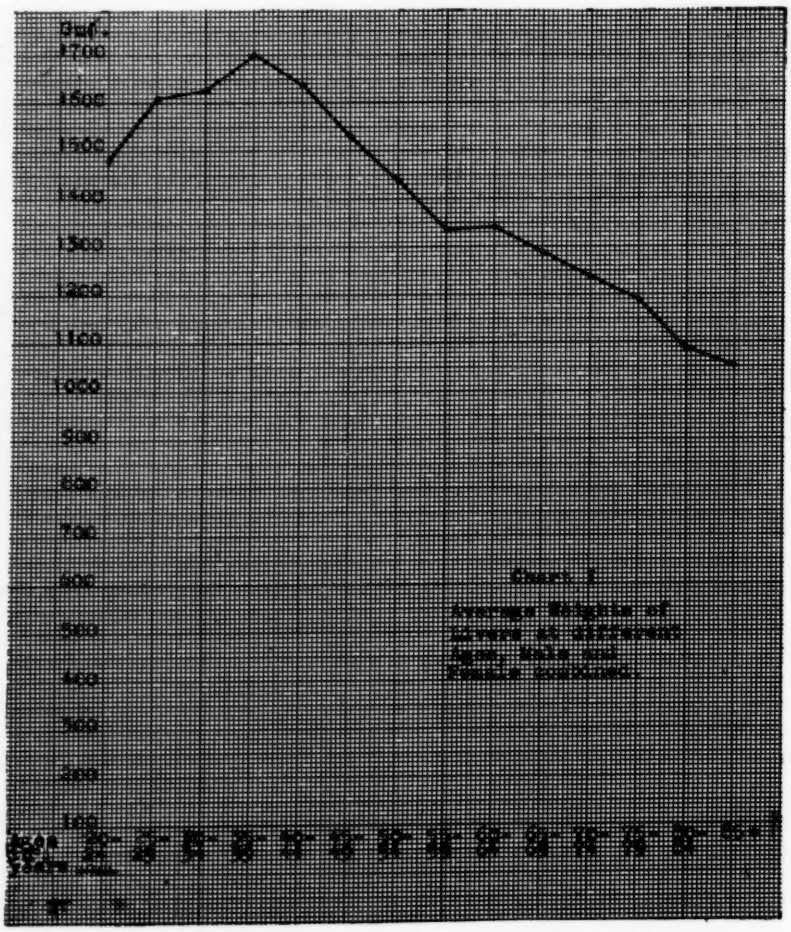

Chart I.

remarkable. Data on the weights of organs at different ages are incomplete and not readily accessible. The average weights given by various authors compiled from cases of adults of all ages are not of much service to a path-

*From the Pathological Laboratory, Long Island Hospital, Boston, Mass.
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ologist as a gauge of what is normal in an elderly individual. The increase in weight during adolescence has been more completely studied than the gradual decline during senescence.

It was, therefore, thought worth while to compile a table and plot a curve of weights according to ages, which might be used as a standard of comparison in the performance of routine autopsies. As an index of senile atrophy of the viscera, the liver was selected as an organ which is less influ-

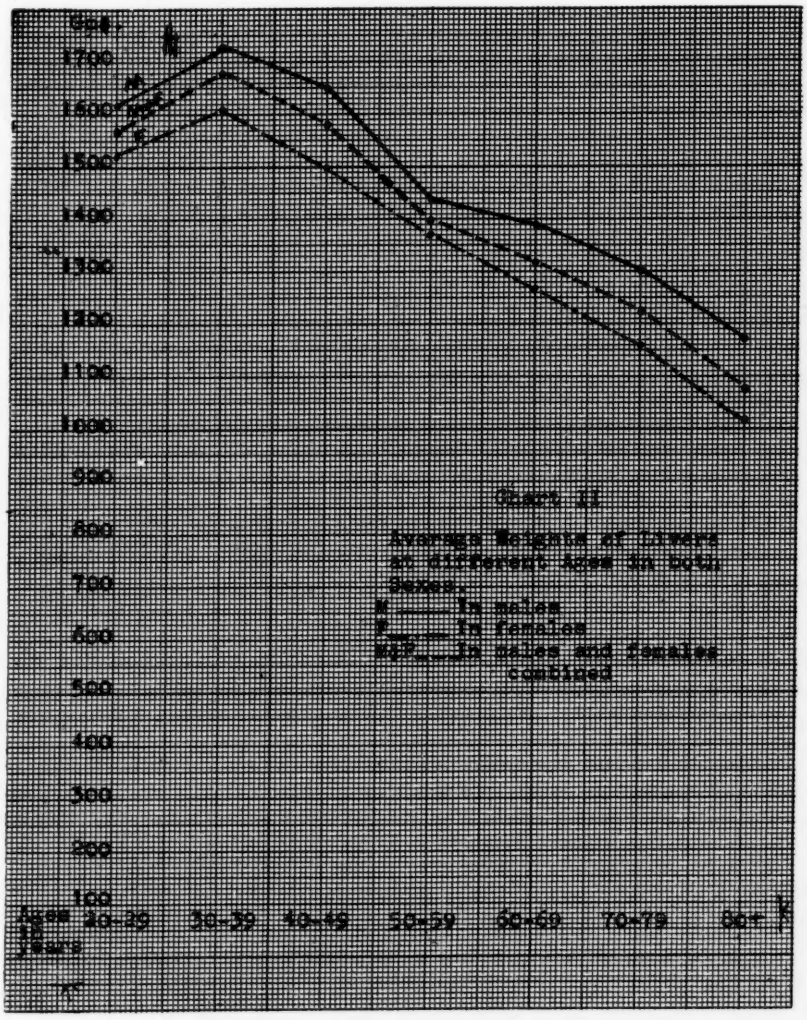

Chart II.

enced by temporary conditions of infection and circulation than the spleen, and as less frequently involved in chronic lesions which would affect the weight than the kidneys and heart. The weights in consecutive autopsies were tabulated according to age, excluding, however, gross lesions of the liver, aside from senile atrophy, which would materially alter the weight, such as cirrhosis, tumors, amyloid change, and abscesses, and omitting cases under twenty years of age.

TABLE I

AVERAGE WEIGHTS OF LIVERS AT DIFFERENT AGES

	20-24 YRS.	25-29 YRS.	30-34 YRS.	35-39 YRS.	40-44 YRS.	45-49 YRS.	50-54 YRS.	55-59 YRS.	60-64 YRS.	65-69 YRS.	70-74 YRS.	75-79 YRS.	80-84 YRS.	85+ YRS.
No. of cases	9	20	32	42	43	45	66	47	88	82	90	49	49	22
Average weight of Liver (gm.)	1482	1612	1627	1704	1638	1533	1441	1339	1346	1290	1239	1186	1087	1054

TABLE II

AVERAGE WEIGHTS OF LIVERS IN THE TWO SEXES AT DIFFERENT AGES

		20-29 YEARS	30-39 YEARS	40-49 YEARS	50-59 YEARS	60-69 YEARS	70-79 YEARS	80+ YEARS
Males	No. of Cases	15	41	50	45	68	64	26
	Average weight of Liver in grams	1620	1733	1650	1435	1388	1301	1174
Females	No. of Cases	14	33	38	68	102	75	45
	Average weight of Liver in grams	1520	1612	1498	1373	1273	1164	1020
Males and Females	No. of Cases	29	74	88	113	170	139	71
	Average weight of Liver in grams	1571	1679	1584	1398	1319	1227	1077

Six hundred and eighty-four cases were studied, of these 309 being in males and 375 in females. In this series the maximum weight of the liver was 2975 grams, the minimum 600 grams. Chart I and Table I show the average weights in males and females combined, grouped in five year periods. It will be seen that the greatest average weight of 1704 grams is attained between 35 and 40 years, after which there is a steady decline in weight to an average of 1054 grams in individuals over 85 years of age.

Chart II and Table II show the average weights in each sex, grouped according to ten year periods. Throughout, the average weight in males is greater than in females, but the two curves run parallel, both reaching the maximum of 1773 grams in males and 1612 grams in females in the third decade, and progressively declining to 1174 grams in males and 1020 grams in females after 80 years of age.

FLOCCULATION REACTIONS AS SUBSTITUTES FOR THE WASSERMANN TEST*

By F. Green, M.D., Montreal, Canada

SOON after the application by Wassermann[1] of the complement-fixation for syphilis, numerous observers with the aim of reaching the same results by simpler methods tried to apply in the case of syphilis, methods already approved in other fields of immunity, e.g., the precipitation and flocculation tests. Fornet[2] early proposed his "ring test," which consists in the formation of a whitish ring at the zone of contact of a luetic with a paretic serum *in vitro*. Porges and Meyer,[3] Elias, Neubauer, Porges and Salomon,[4] and others, using the same principle, tried to obtain similar results by mixing 0.2 c.c. of a 1 per cent solution in distilled water of sodium glycocholate with the same amount of an inactivated serum of a luetic patient. When the test tube containing the substances was left for twenty-four hours at room temperature, a manifest flocculation (precipitate) appeared in the fluid if the serum was luetic. Later, Klausner[5] claimed that the same results could be obtained by simply using 0.6 c.c. of distilled water and 0.2 c.c. of active luetic serum. Notwithstanding all the claims advanced on behalf of these various flocculation reactions, everyone had to agree that they were not to be compared with those obtained by the Wassermann reaction.

Soon after the communication of Porges and Meyer, Hermann and Perutz,[6] having observed that for complement-fixation Browning, Cruikshank and Mackenzie[7] had employed lecithin and cholesterin for their artificial antigens, tried to improve and so render more sensitive the solution of sodium glycocholate of Porges and Meyer by adding to it a certain amount of cholesterin. In fact they used two solutions. In Solution I they had two grams of sodium glycocholate, 0.4 grams of cholesterin and 100 c.c. of alcohol at 95 per cent. Solution II was a 2 per cent solution of sodium glycocholate, which had to be freshly prepared on each occasion at the moment of performing the test. The setting up of the test was very simple: to 0.4 c.c. of the active serum of the patient, 0.2 c.c. of Solution I, diluted 1:20, and 0.2 c.c. of Solution II were added. The test tube with its relative control was shaken, plugged with cotton wool and left at room temperature for 20 hours. After the lapse of that time positive syphilitic sera showed large floccules. According to the authors, out of 134 cases of syphilis 118 gave positive results; of 89 control cases only one gave a slight positive reaction.

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The great simplicity of technic, the economy of material in the test and the fact that it could be used by any general practitioner seemed to indicate that it would supplant the Wassermann reaction. But it was soon discovered that the newly proposed test was not as sensitive as the Wassermann reaction, and that a considerable number of sera reacting negatively with the Wassermann reaction gave positive results with the Hermann-Perutz.*

Other experimenters in the same field, e.g., Sachs and Georgi,[8] Sachs and Altmann,[9] Ternuchi and Toyoda,[10] Jacobsthal,[11] Bruck and Hidaka,[12] Hecht,[13] Meinicke,[14] etc., all tried to improve the Hermann-Perutz reaction, so attractive in its simplicity, and practically everyone proposed a method of his own.

Sachs and Georgi, following the idea of Michaelis[15] (who have found that organ extracts and serum of luetics in a given dilution gave a precipitate) prepared cholesterized organ extracts, such as are now used in the Wassermann reaction. They prepared their extracts from ox-heart muscle, freed from fat and minced finely. They added to it alcohol, in the proportion of 5 c.c. of alcohol to 1 gram of organ. The mixture is kept in the dark at room temperature in a glass-stoppered bottle. After five to six days the alcohol is filtered off in a well stoppered bottle and to the extract 1 per cent cholesterin alcohol solution is added until an optimal degree of solution is reached. As a rule they found that to 100 c.c. of raw alcoholic ox-heart extract 200 c.c. of alcohol and 13.5 c.c. of a 1 per cent alcoholic solution of cholesterin had to be added.

For carrying out the test 0.5 c.c. of this extract, diluted six times with 0.85 per cent NaCl solution, and 1 c.c. of patient's serum inactivated at 55° C. diluted 1:10 with 0.85 per cent NaCl solution, were put in a test tube, incubated at 37° C. for two hours and then taken out of the water-bath and left at room temperature for eighteen to twenty hours. After this time the results are read by means of the Kuhn-Woitke agglutinoscope.

It is understood that the extracts for this reaction as for all similar reactions based on the same principles, have to be titrated beforehand with a number of luetic and normal sera, and the proper dose has to be determined which will give with a certain positive serum a good flocculation and with a negative serum no sign of flocculation. When looked at in the agglutinoscope with a good magnifying lens, a strongly positive serum shows innumerable minute particles or flocculi in suspension in a clear fluid. If the observer has had no previous experience it is very difficult at first to differentiate a positive from a negative serum, and impossible altogether to judge of any borderline case. Furthermore, if the results are read in direct light even with the help of a good magnifying lens, no good readings will be obtained without long practice or the help of the agglutinoscope. The best way in such a case is to compare the serum in question with the control test tubes, which here as in other reactions are also indispensable.

*At the time, 1911, soon after the authors proposed this reaction, along with Dr. H. Hirschfeld of the Charité Krankenhaus in Berlin, I tried the reaction on a number of sera, but the results were very unsatisfactory and as at the time I was engaged in other researches, I abstained from publishing the results.

Sachs and Georgi claimed that in 94.94 per cent of cases their reaction gave the same results as the Wassermann reaction. The Sachs-Georgi flocculation reaction has had a great vogue in Germany and even today in that country many workers claim to get with it results practically as reliable as those obtained by means of the Wassermann reaction. The object of the present paper is to compare the reliability of some of these "short-cut" methods with that of the Wassermann reaction.

THE SACHS-GEORGI REACTION AS COMPARED WITH THE WASSERMANN REACTION

Having prepared a series of three different extracts according to the method given by the authors, and having titrated each of them in different concentrations (keeping the quantity of extract constant, I added alcohol and 1 per cent alcoholic solution of cholesterin in different amounts), I obtained extracts of the following concentrations:

	I	*II*	*III*	*IV*	*V*	*VI*
Raw Alcoholic Extract, in c.c.	1	1	1	1	1	1
Alcohol, in c.c.:	–	1	2	–	1	2
1% Solution of Cholesterin, in c.c.:	0.1	0.1	0.1	0.15	0.15	0.15

In a series of small test tubes each extract was diluted six times, and of this suspension 0.5 c.c. was used. The sera, the one positive and the other negative, were diluted 1:10. In each small test tube there was now 0.5 c.c. of each extract diluted 1:6, and 1 c.c. of the serum diluted 1:10. Each extract had a control tube containing 0.5 c.c. of extract at the same dilution as used for the main test and 1 c.c. of 0.9 per cent NaCl solution. Another control tube contained 1 c.c. of serum diluted 1:10 and 5 c.c. of 0.9 per cent NaCl solution without extract.

Having carried out the same operation for each extract, I put the test tubes in the water-bath at 37° C. for two hours, then took them out and left them at room temperature for two hours. Thereupon the results were read with the agglutinoscope. That dose of the extract was chosen which gave an optimum flocculation with a positive serum, while the test tubes containing the negative serum and the controls, remained absolutely clear, the uniform milky appearance of the emulsion being readily distinguishable from flocculation.

The dose of the extract having been thus determined, I passed to the setting up of the main test, the extreme simplicity of which is no doubt the main advantage of this reaction. For the main test I used two rows of test tubes, the front row contained 1 c.c. of the serum to be examined, diluted 1:10, and 0.5 c.c. of the extract, diluted 1:6. The test tubes in the second row contained 1 c.c. of the same serum as the front row, diluted 1:10, to which, moreover, to make up the same volume as was contained in each of the front row test tubes, 0.5 c.c. of 9 per cent NaCl solution, instead of 0.5 c.c. of extract, was added. The rack containing all the test tubes was now put in the water-bath at 37° C. and left there for two hours, after which time it was taken out and left at room temperature for twenty hours. The reading has to be done either with an agglutinoscope or with an improvised appa-

ratus consisting of a good sized square wooden box, blackened all over and containing an efficient electric lamp. In the front portion of the box a narrow slit, less than half a centimeter wide is left, so that the rays of light are made to pass through it and strike the fluid contained in the test tube on a less than 0.5 cm. space. In this way any slight degree of flocculation in the fluid will be observed. The reading takes place in a dark room or may be carried out in ordinary light under a piece of black cloth. As mentioned before, the use of a hand lens is indispensable.

The two test tubes, one of the front row and one of the second row, are held slightly slanting against the slit in the box. If the serum comes from a luetic patient, the test tube of the first row will show a mass of very fine whitish granules or flocculi, like ground glass, suspended in a clear fluid, while the test tube of the second row containing the serum without extract will show a clear limpid fluid. The reading, as stated, is very hard at times, especially with sera on the borderline, which as usual are exactly those we want to know how to classify.

I have tested more than a hundred sera with this reaction and must venture to say that the reaction is not as sensitive as the Wassermann reaction. The Sachs-Georgi has given me 49 per cent negative results, 43 per cent positive and 8 per cent doubtful. The same sera tested by the Wassermann reaction gave me 28 per cent negative, 68 per cent positive, and 4 per cent doubtful; so that 25 cases with a relatively strong positive Wassermann reaction and with a definite history of syphilis were left undetected by the Sachs-Georgi reaction. The want of correspondence revealed in these results constitutes a serious verdict against the Sachs-Georgi reaction. One fact of interest noted was that the sera giving anticomplementary results with the Wassermann gave also exactly the same result with the Sachs-Georgi, illustrating the fact already noted by Gengou[16] that precipitates will remove complement from a mixture.

THE Σ REACTION OF DREYER AND WARD AS COMPARED WITH THE WASSERMANN REACTION

Quite recently Dreyer and Ward[17] proposed an improvement upon the Sachs-Georgi flocculation reaction, which they recommend not only as a qualitative but also as a quantitative test. They claim that the Σ reaction has given, in their hands, as good results as the Wassermann reaction, that it may be easily standardized and the results expressed in standard units, and that consequently the different methods of treatment may readily be compared one with another.

They work with extracts prepared according to the method of Bordet and Ruelens,[18] which extracts are said to be more stable and to give more reliable results.

The extract of Bordet and Ruelens is prepared so that to 100 grams of calf's heart-muscle, freed from fat and minced very fine, 125 c.c. of 95 per cent alcohol is added and the mixture left at room temperature for seven

days. The mixture, kept in a well stoppered glass bottle, is occasionally shaken. After a week the alcohol is poured off and the organ residue is dried in the incubator at 37° C. for twenty-four hours. When it is well dried 200 c.c. of pure acetone is added and the mixture kept in a well stoppered glass bottle in the incubator at 20° C. for seven days. The acetone is then filtered off and the residue is again treated with another 100 c.c. of acetone and kept at 20° C. for one day. The acetone is again filtered off and the residue is dried in the incubator at 20° C. for two hours, whereupon 200 c.c. of 95 per cent alcohol is added to the residue. This mixture is put in a glass-stoppered bottle in the incubator at 20° C. for ten days and then filtered through filter paper. The filtrate, which contains the acetone-insoluble, alcohol-soluble substances of the heart-muscle, is kept in the dark at room temperature in a well stoppered glass bottle and is ready for use.

Besides the extract, a 1 per cent alcoholic solution of cholesterin is prepared and kept under the same conditions.

From the extract they prepare two heart-cholesterin saline suspensions, which they call α and β suspensions. For the preparation of α suspension they pipette into a clean, dry test tube 5 c.c. of the extract and 0.25 c.c. of the cholesterin solution and mix them. From this, with a clean pipette, 1 c.c. of the heart-cholesterin extract is carefully pipetted into a 100 c.c. measuring cylinder and the fluid is left running out of the pipette when this has touched the bottom of the cylinder, without letting the extract come in contact with the wall of the cylinder. Then 10.7 c.c. of a 0.9 per cent NaCl solution is dropped in (dropping distance 36 cm.) and the NaCl solution dropped at a constant rate by a special mercury pressure dropping apparatus described by the authors and which is very easily constructed. Afterwards, without shaking, the resulting suspension is gently mixed by inverting the cylinder several times. (This slow method of preparing the suspensions is the same as is used in the Sachs-Georgi and also in the Wassermann reaction). This represents α suspension. Suspension β is prepared in a like manner, only instead of 10.7 c.c., 34 c.c. of NaCl solution is added.

Two dilutions of the patient's serum are now prepared, one 1:10, the other 1:20.

I have tested the Dreyer and Ward reaction only qualitatively.

In a specially constructed brass rack nine agglutination test tubes (6 in. long by 0.6 in. internal diameter) with conical end were placed. In the first five I put a different number of drops of the serum diluted 1:10; in test tube I, 20 drops; in II, 10 drops; in III, 5 drops; in IV, 2 drops and in V, 1 drop. In the remaining four test tubes I put as many drops of the same serum but diluted 1:20, viz.: in test tube VI, 10 drops; in VII, 5 drops; in VIII, 2 drops and in IX, 1 drop. Into test tube I, I put 6 drops of α suspension, into the remaining eight test tubes 15 drops of β suspension. NaCl solution is now added, drop by drop, to each test tube until each, with the exception of the first which already contains 26 drops, contains 25 drops altogether.

The dilutions in this way vary from 1/1.25 to 1/462. A control tube containing 20 drops of NaCl solution and 6 drops of α suspension, and another control tube containing 10 drops of NaCl solution and 15 drops of β suspension, are placed in the same rack. After each test tube has been well shaken, the rack containing the test tubes is put in a water-bath set at 37° C. and left there for seven hours. After this long incubation the rack is taken out and left at room temperature for 10 minutes, whereupon the results are read in the same way as mentioned before in the case of the Sachs-Georgi. All the various precautions advised by the authors (for details see their paper) were carefully observed. Having examined each serum in the dilution of 1:10 and 1:20, and having found that positive results are very rarely obtained with dilutions of 1:20 (even then they are inessential for merely qualitative tests), I carried on the research solely with the first 5 test tube dilutions. The Σ reaction was tried on more than 150 sera and the results were in each case compared with those given by the Wassermann reaction. The percentage obtained is the following: 34 per cent negative, 58 per cent positive, 8 per cent doubtful. As already stated in an earlier part of this paper with the Wassermann reaction carried out on the same sera I obtained 28 per cent negative, 68 per cent positive, 4 per cent doubtful. Ten sera which gave a positive Wassermann reaction *responded negatively with the Σ reaction.* When now the three reactions, the Wassermann, the Sachs-Georgi and the Dreyer-Ward are compared, it is found that the first is the most sensitive of the three and the second quite unreliable, while in view of its simplicity and the ease with which it may be standardized the Dreyer-Ward reaction, granted an increase in its sensitivity and improvement in the matter of reading the results, might in future very well stand alongside the Wassermann reaction.

I have to extend my best thanks to Dr. L. Notkin of this Department for having helped me in the first stage of the Dreyer-Ward reaction.

BIBLIOGRAPHY

[1]Wassermann, Neisser and Bruck: Deutsch. med. Wchnschr., 1906, No. 19.
[2]Fornet: München. med. Wchnschr., 1908. lv, 161.
[3]Porges O. and Meyer, G: Berl. klin. Wchnschr., 1908, xlv, 731.
[4]Elias, H., Neubauer, E., Porges, O., and Salomon, H.: Wien. klin. Wchnschr., 1908, No. 23, 831.
[5]Klausner, E.: Wien. klin. Wchnschr., 1908, xxi, 214.
[6]Hermann, O. and Perutz, A.: Med. Klin., 1911, No. 2.
[7]Browning, C. H., Cruickshank, J., and Mackenzie, J.: Biochem. Ztschr., 1910, xv.
[8]Sachs, H. and Georgi, W.: Med. Klin., 1918, No. 33.
[9]Sachs and Altmann: Berl. klin. Wchnschr., 1908, No. 14.
[10]Ternuchi, Y. and Toyoda, H.: Wien. klin. Wchnschr., 1910, xxiii, 919.
[11]Jacobsthal, E.: Ztschr. f. Immunitätforsch. und exper. Therap., 1910, viii, 107.
[12]Bruck, C. and Hidaka, S.: Ztschr. f. Immunitätsforsch. und exper. Therap., 1910-11, viii, 476.
[13]Hecht, H.: Ztschr. f. Immunitätforsch. und exper. Therap., 1910-11, 1, 433.
[14]Meinicke, Berl. klin. Wchnschr., 1917.
[15]Michaelis, L.: Berl. klin. Wchnschr., 1907, No. 46, 1477.
[16]Gengou, O.: Ann. de l'Inst. Pasteur, 1904, xviii, 678; also Ztschr. f. Immunitätsforsch und exper. Therap., 1911, xxx, 154.
[17]Dreyer, G. and Ward, H. K.: The Lancet, May 7, 1921.
[18]Bordet, J. and Ruelens, G.: Compt. rend. Soc. de biol., February, 1919, lxxxii.

A COMPARATIVE STUDY OF THE WASSERMANN AND MEINICKE REACTIONS*

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SINCE the great value of the Wassermann reaction in the diagnosis of syphilis has become generally recognized the test is indicated in all suspected cases of this disease. With many physicians it is a routine procedure during the investigation of obscure symptoms, and furthermore, as a means of controlling treatment, the test is of the greatest importance. It is doubtful if any method of laboratory diagnosis has been more carefully studied than the Wassermann reaction. Certainly no other laboratory test has been subjected to so many attempts at modification for the purpose of simplifying the technic and rendering the results more accurate.

Many modifications of the original Wassermann technic have been proposed. Nonspecific antigens, prepared in various ways from various organs of animals of different species, and in some cases fortified by the addition of lipoidal substances, are now in general use. The results, when such antigens are used, are certainly as reliable as the results obtained from the use of antigens prepared from syphilitic tissue according to Wassermann's original method. The antisheep hemolytic system is perhaps the choice of most laboratory workers, while guinea pig serum as complement is in general use throughout the world. Although antigen and amboceptor are within certain limits permanent they must be titrated immediately before performing the test. Methods have been proposed by which complement and sheep cells may be preserved for a period of one or two weeks, but many question the efficacy of these methods of preservation. Well recognized sources of error are the presence in certain serums of anticomplementary substances and natural hemolysins.

The Wassermann test, however it may be modified, requires a number of reagents and the standardization of at least three of these immediately before use. Since the preparation and standardization of the various reagents requires considerable time as well as the possession of rather elaborate equipment and a supply of laboratory animals, a reaction as reliable but more simple in execution would be a welcome addition to our methods of laboratory diagnosis.

Immediately after Wassermann's original publication the serum of syphilitic individuals became a popular field of research. It was soon recognized that the Wassermann reaction was not an example of the fixation of complement as a result of the interaction of specific antigen, specific serum, and

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complement. Furthermore, it was demonstrated that lipoidal substances played a rôle in this reaction. During this early period of research a number of methods for the serum diagnosis of syphilis were proposed. These methods were based upon the principle that precipitation occurs when syphilitic serum is brought in contact with certain substances. Among the substances proposed may be mentioned distilled water, suspension of lecithin, suspension of sodium glycocholate, suspension of sodium glycocholate plus cholesterin. None of these methods have come into general use.

More recently two flocculation reactions have been proposed which give promise of assuming a permanent place among laboratory methods, namely the Sachs-Georgi[1] reaction and the Meinicke[2] reaction (third modification). It is the object of this paper to present the results of a comparative study of the Wassermann reaction and the Meinicke reaction in a series of 1162 cases. The principle of the Meinicke reaction may be stated briefly as follows: Normal serum when mixed with a saline emulsion of an alcoholic extract of horse heart, which has been previously extracted with ether, prevents the spontaneous precipitation of the lipoids contained in the extract, whereas syphilitic serum hastens the precipitation and at the same time increases the size of the floccules.

It may be mentioned here that for the Wassermann tests the antisheep hemolytic system and beef heart antigen with and without the addition of cholesterin were used. The Meinicke reaction was carried out according to Meinicke's directions, except a 3 per cent sodium chloride solution was substituted for a 2 per cent sodium chloride solution, and to the antigen there was added one half its volume of glycerine just before adding the salt solution. The addition of glycerine to the antigen was made because its presence seemed to make the reaction more marked by increasing the size of the floccules many of which remain in suspension. This fact was noted after testing a mixture of several syphilitic serums which had been preserved by the addition of an equal volume of glycerine. When tested for the first time this pooled serum was eight months old and gave a strong positive Meinicke reaction and showed the presence of unusually large floccules. Such large floccules had not been observed in other positive serums, and their presence could only be due to the presence of glycerine or to the age of the serums. Further experiment showed that glycerine produced this result and as a result of this observation the addition of glycerine became a part of the routine procedure.

The serums to be tested were obtained from Wesley Hospital, from the dispensary of Northwestern University Medical School and from private sources. The Wassermann readings were made by four different individuals. In only a small number of cases was it possible to obtain clinical data and the primary object of this study was to compare the results obtained with those obtained with the Wassermann reaction.

Before discussing the comparative results of the two reactions a descrip-

tion of the method of preparation and standardization of the antigen, and the technic of the test will be given.

PREPARATION OF LIPOID EXTRACT

Several horse hearts are freed from fat, vessels and connective tissue and hashed in a meat chopper. The hashed heart muscle is then spread out on a glass plate in a thin layer, allowed to dry for several days at incubator temperature, and then ground to a fine powder. To one part of this powder nine parts of ether are added and the mixture shaken for one hour. The ether is then poured off and the powder washed again with ether, after which it is filtered and the residue allowed to dry. One part of the dried powder is now mixed with nine parts of 95 per cent alcohol and extracted for several days at 37° C., shaking several times daily. The extract should be kept at room temperature since a precipitate forms when the extract is kept at ice box temperature. Before use the extract must be standardized after which no further titration is necessary.

STANDARDIZATION OF EXTRACT

Meinicke has proposed the following method of determining the strength of the extract to be kept as a stock solution.

Decreasing amounts of the alcoholic extract are mixed with increasing amounts of 95 per cent alcohol as follows:

0.5 c.c.	0.45 c.c.	0.4 c.c.	0.35 c.c.	0.3 c.c.	0.25 c.c.	Extract.
0	0.05 c.c.	0.1 c.c.	0.15 c.c.	0.2 c.c.	0.25 c.c.	Alcohol.

The tubes are well shaken and 0.25 c.c. of distilled water added to each tube and the tubes again shaken. They are allowed to stand for an hour at room temperature after which 3.5 c.c. of distilled water are added to each. The proper concentration of extract is in the tube in which immediately after the first addition of water turbidity appears, and which during the one hour period becomes milky in appearance, and on the second addition of water clears somewhat, but still remains distinctly turbid. If the concentration is too strong the mixture becomes milky opaque immediately after the first addition of water, while a too weak concentration becomes almost entirely clear after the second addition. The proper concentration having been determined the extract is diluted according to the following method: For example, the proper concentration is present in the tube containing 0.3 c.c. extract plus 0.2 c.c. alcohol. To 30 parts extract add 20 parts of 95 per cent alcohol and mix well. This is the stock extract from which the saline emulsions are prepared immediately before addition to the serum to be tested. The extract may also be tested by adding directly to known positive serums a mixture of alcohol and extract in the above proportions plus 2 per cent or 3 per cent sodium chloride solution as described under the method of performing the test.

DILUTION OF ANTIGEN

Before use the antigen is diluted by the addition of distilled water and 3 per cent sodium chloride solution. One-tenth c.c. of the standardized ex-

tract is used for each test. After determining the amount of extract to be used and measuring it into a flask one-half this amount of distilled water is added, the flask well shaken and allowed to stand for an hour at room temperature. The final step in preparing the antigen is the addition of 3 per cent sodium chloride solution in an amount seven times that of the amount of alcoholic extract used for the series. The extract should be added immediately to the serum since spontaneous flocculation occurs in a relatively short time after the addition of the salt solution.

THE TEST PROPER

Two-tenths c.c. of each serum to be tested are placed in small glass tubes and 0.8 c.c of the diluted antigen are added to each. The tubes are well shaken and placed in the incubator where they should remain at least twelve hours before reading. The incubator temperature should not be higher than 37° C. Many cases show a definite positive reaction after twelve hours incubation, but the final reading should be made after twenty-four hours incubation and another twenty-four at room temperature. A positive reaction resembles a more or less pronounced agglutinative reaction characterized by the presence of small clumps which are generally visible to the naked eye. In well marked reactions a precipitate appears at the bottom of the tube and the supernatant fluid is quite clear. Although many positive reactions may be recognized macroscopically it is preferable to make the readings with the aid of a hand lens. Many specimens show a finely granular appearance resembling a dilute bacterial suspension. These do not represent true flocculation and are readily distinguished from a positive reaction.

The comparative results obtained by the Wassermann reaction and the Meinicke reaction are shown in Tables I and II.

TABLE I

REACTIONS WITH SERUM	NO.	PER CENT
Wassermann positive, Meinicke positive	149	12.82
Wassermann negative, Meinicke negative	998	85.88
Total reactions in agreement	1147	98.7
Wassermann positive, Meinicke negative	10	.86
Wassermann negative, Meinicke positive	5	.43
Total reactions not in agreement	15	1.29
Total number	1162	

From Table I it will be seen that the two reactions agree in 98.7 per cent of the cases studied. Serums which gave a +++ Wassermann and a ++ Wassermann have been included with the positive reactions in agreement and are discussed under Table II.

TABLE II

POSITIVE REACTIONS IN AGREEMENT

WASSERMANN	MEINICKE	NO.
++++	+	125
+++	+	16
++	+	8

Of the 125 cases which gave a ++++ Wassermann and a positive Meinicke reaction the clinical diagnosis could be obtained in only a small per cent. A diagnosis of syphilis was made in 21 cases and the following diagnosis was given in 6 cases: Organic heart disease, old tuberculosis, otosclerosis, arteriosclerosis, venereal warts, repeated miscarriages.

Of the 16 cases which gave a +++ Wassermann and a positive Meinicke one case was diagnosed as syphilis, one case as syphilis (?) and in 14 cases no diagnosis could be obtained.

The remaining tests which are in agreement comprise a series of 8 cases showing a ++ Wassermann and a positive Meinicke. No diagnosis was stated in 6 cases while the remaining 2 cases were definitely diagnosed as syphilis.

TABLE III
REACTIONS NOT IN AGREEMENT

NO.	WASSERMANN	MEINICKE	CLINICAL DIAGNOSIS
1	+++	−	No diagnosis
1	+++	−	"
1	+++	−	"
1	++	−	"
1	++	−	"
1	++	−	"
1	++	−	"
1	++	−	"
1	++	−	"
1	++	−	Syphilis (?)
1	Anticomplementary	+	Syphilis. Beginning tabes.
1	−	+	Syphilis
1	−	+	Syphilis. Undergoing treatment.
1	−	+	Syphilis
1	−	+	Syphilis
Total 15			

From Table III it will be noted that 10 cases show a positive Wassermann and a negative Meinicke reaction. These, however, comprise only cases giving a +++ or ++ Wassermann. Unfortunately a diagnosis could be obtained in only one case of this group and in this single instance the diagnosis was questionable.

The remaining 5 cases are of interest since it is from this group that certain conclusions may be made. The diagnosis in these 5 cases was definitely stated to be syphilis. It is to be noted that 4 of the cases gave a negative Wassermann and that the fifth case was anticomplementary, while every case with a clinical diagnosis of syphilis gave a positive Meinicke reaction.

SUMMARY

The most striking features of this series of cases are: First, the high per cent of cases in which the two reactions agree; second, the superior results obtained by the Meinicke test in a small group in which the diagnosis of syphilis was unquestioned. In the absence of autopsy findings or histologic proof the clinical diagnosis is the ultimate criterion of the value of any laboratory test; therefore, any test which is to be regarded as specific should be based on such findings rather than on a comparison with another test.

In this series it was possible to obtain clinical data in only a small per cent of the cases examined, but when such data was at hand it furnished conclusive evidence as to the value of the Meinicke reaction.

CONCLUSIONS

1. The Meinicke reaction agrees with the Wassermann reaction in more than 90 per cent of serums.

2. The Meinicke reaction provides a simple and reliable flocculation method for the examination of suspected serums.

3. The Meinicke reaction is positive in certain cases which are negative by the Wassermann reaction.

REFERENCES

[1]Sachs and Georgi: Med. Klin., 1918, No. 33. S. 805.
[2]Meinicke: München med. Wchnschr., 1919, No. 33.
Epstein and Paul: Med. Klin., 1920, No. 19.
Hubschmann: München med. Wchnschr., 1920, No. 9.
Kirschner and Segall: Wien. klin. Wchnschr., 1920, No. 18.
Schmidt and Pott: Deutsch. med. Wchnschr., 1920, No. 19.
Blasius: Deutsch. med. Wchnschr., 1920, No. 31.
Pesch: München. med. Wchnschr., 1920, No. 43.
Weibach: Deutsch. med. Wchnschr., 1921, No. 22.
Heinemann: München. med. Wchnschr., 1921, No. 48.
Schneider: Berl. klin. Wchnschr., 1921, No. 51.
Winkler: Med. Klin., 1922, No. 4.
Bering: Med. Klin., 1922, No. 32.

THE RECIPROCAL RELATIONSHIP OF CHLORIDES AND GLUCOSE IN THE BLOOD*

By W. W. Herrick, M.D., New York City

IN the course of several routine glucose tolerance tests on a group of office patients, it was suggested by the chemist, Mr. J. W. Nerses, that at the same time determination of the chlorides of the whole blood be made. This was done and the curves showing the simultaneous concentration of these substances in the whole blood were plotted on the same charts. The cases were miscellaneous medical conditions quite unselected. The patients reported at the office in the morning without having taken breakfast, blood was taken from the vein, 100 grams of glucose was then given by mouth in solution and subsequent specimens of blood were withdrawn at the time intervals indicated on the individual charts. For the determination of glucose the ordinary Folin-Wu method was used. Chlorides were determined by the Whitehorn method.[1] The striking results are best illustrated by the attending charts. That there is a fairly constant reciprocal relation between the concentration of these crystalloids in the blood under the conditions of the ordinary glucose tolerance test in a fasting patient seems clear. In the five

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unselected examples taken from consecutive cases, only minor variations are apparent.

In the chart of Case I, in which the curve for glucose is of the so-called diabetic form, the curve denoting the concentration of chlorides returns to its former level before that for glucose sinks. In the remaining examples the inverse linear relationship is in general maintained.

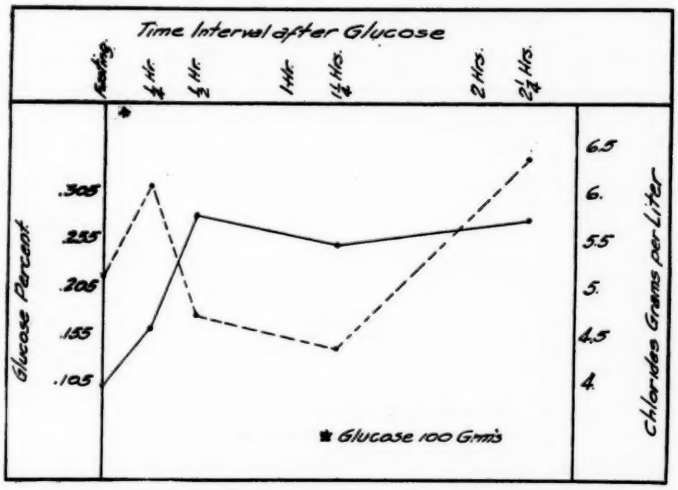

Chart 1.—(Case 1.) Woman, aged 35, suffering severe headache with symptoms suggesting a vascular cerebral tumor. Diagnosis not established.

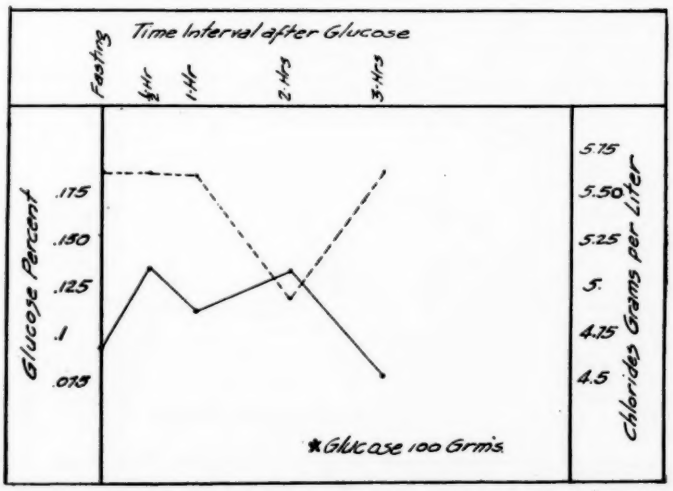

Chart 2.—(Case 2.) Boy, aged 16, suffering progressive loss of vision from retrobulbar neuritis without other symptoms. No cause found.

The significance of this finding is not altogether clear. However, a number of interesting speculations are at once suggested. Some of these have been touched upon by others. McLean[2] states that there is a "diminution of the chlorides in the plasma of nephritics during life up to the time of death in uremic coma." This writer states his belief that such diminu-

tion in chlorides in the plasma is a normal accompaniment of uremia and particularly of the "increased H-ion concentration frequently observed in uremic patients shortly before death." In view of other findings, it may also be asked if the increase of the nonprotein nitrogen, which occurs almost uniformly under the given conditions, may not also be an influence.

McLean, Murray and Henderson,[3] found that "on plotting the serum

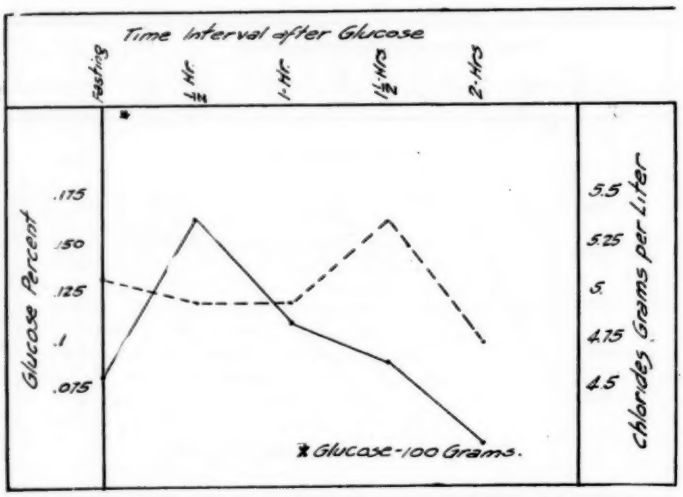

Chart 3.—(Case 3.) Man, aged 70. Cerebral arteriosclerosis.

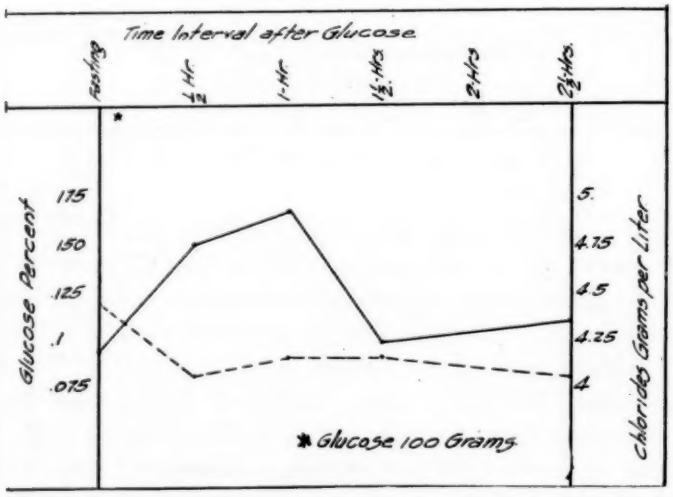

Chart 4.—(Case 4.) Man, aged 23. Asthenia and arterial hypotension.

bicarbonate against the serum chlorides," they had a linear relationship. "An increase in the concentration of bicarbonate in the serum is accompanied by a decrease of chlorides corresponding to somewhat more than one-half of the increase in bicarbonate."

Probably the simplest and most attractive explanation of this behavior of the chlorides in the presence of increasing concentration of other crys-

talloids in the blood is that, in an attempt on the part of the body to maintain its fluids in osmotic balance, the chlorides are forced out. The low plasma chlorides found in certain cases of diabetes may thus be accounted for as an attempt to preserve osmotic balance. This forcing out of the chlorides might be by excretion or by redistribution, that is, by increased

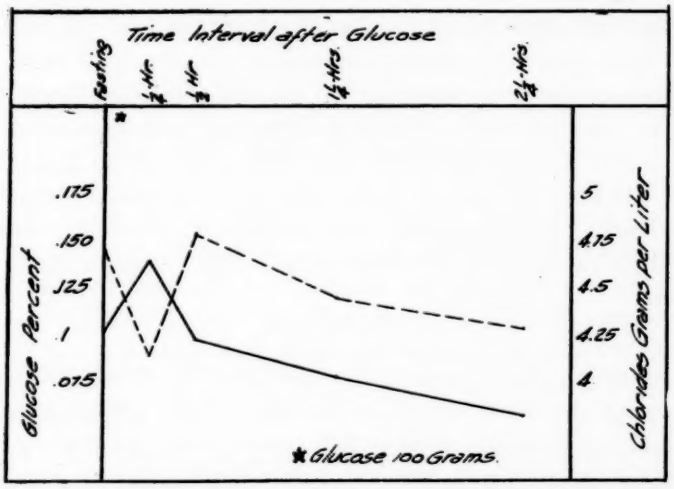

Chart 5.—(Case 5.) Man, aged 42, chronic iritis, failing vision. Cause not determined.

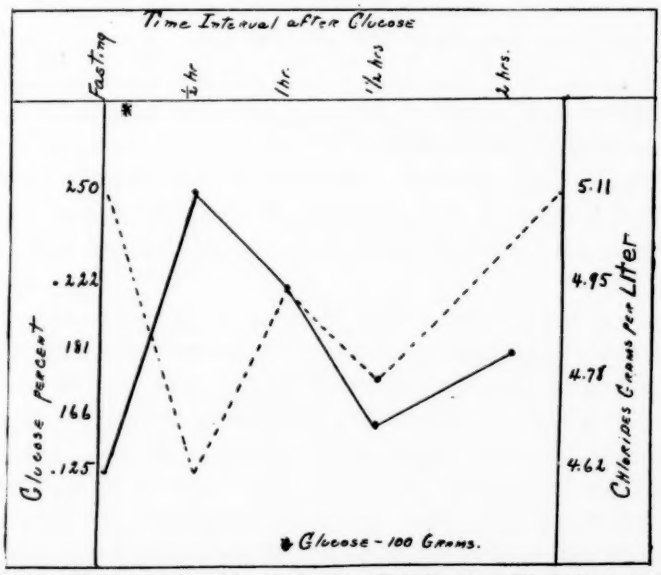

Chart 6.—(Case 6.) Woman, aged 24. Endocrine disturbance with heterosexual characteristics; possible tumor of adrenal cortex.

concentration in other tissues. Atchley[4] suggested that the chlorides might be forced from the serum into the corpuscles of the blood by change in the carbonate concentration, the Zunst reaction.[5] It is to be doubted that the latter explanation can apply to the consequences to the chlorides of changes in the concentration of glucose in the blood, since our determinations were

made with the whole blood. Again, one may raise the question whether the phenomenon is not a dilution rather than a replacement. In this the matter of blood volume becomes a vital speculation. In general, the chlorides seem the most readily mobilizable of any of the observed crystalloids. Accommodatingly they appear to give place to glucose, the carbonates, the retained urea and doubtless to other similar compounds. It may be that they are the chief regulators of osmosis in the body.

It has been asserted, but by no means proved, that there is a direct relation between the concentration of chlorides in the blood and arterial hypertension. Certain cases of hypertension are also associated with hyperglycemia. There are relationships here that may be as important as they are obscure. It may be that the debatable factor of blood volume in relation to blood pressure is concerned. The weight of evidence, however, is at present against the view of a causal relationship between blood volume and hypertension.

CONCLUSION

There is an inverse relationship between the concentration of glucose and chlorides in the blood after the ingestion of 100 grams of glucose. This suggests that the chlorides possess the property of shifting to other tissues from the blood, in order to preserve the optimal osmotic conditions in the blood.

REFERENCES

[1]Whitehorn, J. C.: Jour. Biol. Chem., 1921, xlv, 449.
[2]McLean, F. C.: Proc. Soc. Exper. Biol. and Med., N. Y., 1916, xiii, 166-168.
[3]McLean, F. C., Murray, H. A., Henderson, L. J.: Proc. Soc. Exper. Biol. and Med., 1919-20, xvii, 180.
[4]Atchley, D. W.: Arch. Int. Med., 1918, xxii, 405.
[5]Zunst: Ztschr. Physiol. d. Bonn, 1868.

LABORATORY METHODS

TECHNIC FOR THE BACTERIOLOGIC DIAGNOSIS OF BACILLARY DYSENTERY*

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IN the south, where the summers are long and intense and the winters are comparatively mild, dysentery, although probably not epidemic in many sections, is certainly endemic to a degree of intensity not generally recognized. This, coupled with the fact that there is much diarrhea and enterocolitis of unknown etiology, and which simulates dysentery clinically, makes a reliable and rapid differential bacteriologic diagnosis a highly desirable aid to the clinician's observations.

With such a purpose in view the problem of arriving rapidly at a bacteriologic diagnosis by a study of the feces was undertaken. The attending difficulties are of course great, and the utmost care must be taken to obtain satisfactory results. However, the pressing need for such a diagnostic method stimulated trial and the following routine procedure was developed.

1. Initial plating of feces, or preferably mucus from ulcers taken with a proctoscope and swab, on eosin-methylene blue agar containing 0.5 per cent each of lactose, saccharose and glycerine. Reaction adjusted to pH 7.2.

2. Pick suspected colonies. Transplanting at least three to Krumweide's triple sugar tubes for the purpose of checking the initial diagnosis and identifying the type.

3. At the time emulsify a mixture of several colonies in a drop of 1 to 30 polyvalent dysentery serum, mounted for a slide agglutination. Observe agglutination over a period of one hour. Partial agglutination at the end of that time is considered presumptive diagnosis of dysentery.

4. Transplant growth from the triple sugar tubes to dextrose, maltose, mannitol and sucrose in agar with brom-cresol-purple as the indicator and observe acid formation for 48 to 72 hours. The purpose of the long period on the last step is to give time for the Flexner strains to ferment maltose. Even then the fermentation of this sugar is practically always slight.

The purpose of the saccharose and glycerine added to the eosin methylene blue agar is to exclude from the probable dysentery colonies, those several intestinal bacteria that are nonlactose fermenting organisms, but that do ferment the added sugars. (This procedure renders the medium

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unfit for the isolation of Eberth. typhi and Salmonella paratyphi as both these organisms ferment glycerine.)

Before such a technic could be accepted as a reliable procedure for the isolation and identification of the dysentery organisms, certain points must be supported experimentally. In the first place the selective ability of the modified medium must be proved. To this end thirteen strains of Eberth. dysenteriae and para dysenteries were plated on the modified and the unmodified medium, as indicated in Experiment I. The results show that

EXPERIMENT I

ORGANISM	SOURCE	CHARACTER OF COLONIES	
		EOSIN-METHYLENE BLUE AGAR	EOSIN-METHYLENE BLUE AGAR MODIFIED
Eberth. paradysenteriae. (Flexner)	Stock	Colorless	Colorless
Eberth. paradysenteriae. (Flexner)	Army Med. School	Colorless	Colorless
Eberth. paradysenteriae. (Hiss)	Stock	Colorless	Colorless
Eberth. paradysenteriae. (Hiss) four strains	Active cases	Colorless	Colorless
Eberth. paradysenteriae. (Hiss)	Army Med. School	Colorless	Colorless
Eberth. dysenteriae	Stock	Colorless	Colorless
Eberth. dysenteriae Three strains	Active cases	Colorless	Colorless
Eberth. dysenteriae	Army Med. School	Colorless	Colorless
Salmonella morgani	Stock	Colorless	Colorless
Salmonella morgani Two strains	Active cases	Colorless	Colorless
Eberth. typhi	Stock	Colorless	Colored
Eberth. paratyphi	Stock	Colorless	Colored
Salmonella schotmulleri	Stock	Colorless	Colorless
Seven unidentified strains	Normal feces	Colorless	Colored
Two unidentified strains	Normal feces	Colorless	Colorless

This experiment is designed to determine, first, that strains of the dysentery group of organisms will remain colorless on the modified medium; and second, that many strains of nonlactose fermenting organisms that are not dysentery will produce colonies on the modified medium that are colored.

all the strains gave colorless colonies on both media. It will also be noted that two strains of Salmonella morgani are included in the experiment, and twelve other strains of nonlactose fermenting organisms, among which are Eberth. typhi, Salmonella paratyphi and Salmonella schotmulleri. Of these twelve strains only three gave colorless colonies on the modified medium, thus showing that many of the colorless colonies on the unmodified medium are colored on the modified agar. The modified medium thus proves to be more selective for the dysentery group than the old medium, and at the very first step in the identification eliminates many confusing colonies from consideration.

The use of the modified medium is not justified, however, unless the lactose fermenting strains of organisms that are not in the dysentery group are

sufficiently numerous to be troublesome in the picking of suspected colonies. Although the large number of such organisms had been noted previously in practice, a series of twelve normal feces samples were plated on both the modified and the unmodified medium to determine this point experimentally. The results are shown in Experiment II. Only two samples showed no fewer colorless colonies on the modified medium than on the unmodified medium. The other samples showed a marked decrease in the number of colorless colonies on the modified medium. This experiment shows that the modified medium actually performs a service in the elimination of many colonies as belonging to the suspected dysentery group, thus facilitating the rapid identification of such members of the group that may be present.

The fundamental point of the technic, however, is the slide agglutination. To establish the value of this step Experiments III and IV were carried out.

EXPERIMENT II

SAMPLE NO.	CHARACTER OF COLONIES ON THE MODIFIED MEDIUM	CHARACTER OF COLONIES ON EOSIN-METHYLENE BLUE AGAR
1	Few colorless colonies	Many colorless colonies
2	No colorless colonies	Many colorless colonies
3	Almost entirely colored	Almost entirely colorless
4	No colorless colonies	About 50% colorless colonies
5	Few colorless colonies	Few colorless colonies
6	Occasional colorless colony	Occasional colorless colony
7	No colorless colonies	No colorless colonies
8	No colorless colonies	Many colorless colonies
9	No colorless colonies	Occasional colorless colony
10	Few colorless colonies	Many colorless colonies
11	No colorless colonies	Few colorless colonies

This experiment is designed to show that in normal feces there are many strains of organisms that will give confusing colonies on plates where the dysentery group is being sought for.

Experiment III shows the result of agglutination studies on nine strains of dysentery organisms. The serum used was a polyvalent stock and was diluted from 1-5 to 1-4000 as shown in the table. This study also includes the two strains of Salmonella morgani. All the strains of dysentery gave complete agglutination in dilutions as high as 1-50 of the serum, the clumping occurring in the first five minutes. The two strains of Salmonella morgani, although they agglutinated in as high dilution as the dysenteries, required at least forty-five minutes for the clumping to become complete. The agglutination was carried out here as in the routine method, that is, in a hanging drop preparation.

In practice the amount of agglutination designated as partial in the table was considered as diagnostic. This is because the number of organisms emulsified in the serum cannot be accurately measured, and it is conceivable that the dose may sometimes be so heavy that all the specific agglutinins would be absorbed before the total number of organisms was clumped. The fact, too, that in practice several colonies are selected and emulsified in one drop may sometimes lead to the mixing of an organism of the dysentery group and some other organism that would not be agglutinated at all.

The experimental evidence in favor of the slide agglutination would

EXPERIMENT III

STRAIN OF THE DYSENTERY GROUP USED	DILUTION OF STOCK POLYVALENT ANTIDYSENTERY SERUM																		
	1 TO 5	1 TO 10	1 TO 20	1 TO 30	1 TO 40	1 TO 50	1 TO 60	1 TO 70	1 TO 80	1 TO 90	1 TO 100	1 TO 200	1 TO 300	1 TO 400	1 TO 500	1 TO 1100	1 TO 1500	1 TO 2000	1 TO 4000
Hiss	C	C	C	C	C	C	C	C	C	P	P	F	F	F	O	O	O	O	O
Hiss	C	C	C	C	C	C	C	P	P	P	P	F	F	F	F	F	F	O	O
Hiss	C	C	C	C	C	C	C	C	C	P	P	F	F	F	F	F	F	O	O
Hiss	C	C	C	C	C	C	P	P	P	P	F	F	F	F	O	O	O	O	O
Dysenteriae (Shiga)	C	C	C	C	C	C	C	C	C	C	C	P	P	P	F	F	F	F	F
Dysenteriae (Shiga)	C	C	C	C	C	C	C	C	C	C	C	P	P	F	F	F	F	O	O
Dysenteriae (Shiga)	C	C	C	C	C	C	P	P	P	P	F	F	O	O	O	O	O	O	O
Flexner	C	C	C	C	C	C	C	P	P	F	F	F	O	O	O	O	O	O	O
Salmonella morgani	C	C	C	C	C	C	C	C	P	P	F	F	F	O	O	O	O	O	O
Salmonella morgani	C	C	C	C	C	C	P	P	P	P	F	O	O	O	O	O	O	O	O
Hiss	C	C	C	C	C	C	C	C	P	P	F	F	O	O	O	O	O	O	O

This experiment is designed to determine the highest dilution of serum that may be used under the conditions of the test to secure complete agglutination of the organisms. *C* indicates complete agglutination; *P*, partial agglutination; and *F*, faint agglutination.

not be complete however unless the possibility of false agglutinations by nondysentery strains of organisms is excluded. During the course of this work only four strains of organisms were encountered that gave characteristic dysentery colonies on the modified medium, and did not on subsequent study prove to be such. Experiment IV shows the result of an agglutination study on these four strains. These strains are designated as strains 1, 2, 3, 4, in the table. In only one instance, strain 3, did any agglutination occur and that only faint in undiluted serum. Five other strains of organisms isolated from normal feces, giving colorless colonies on the modified medium failed to show any agglutination. Many other organisms were tested during the course of the work, and in only one case was a false agglutination observed. Many organisms have been reported by various workers that gave false agglutination in dysentery serum, but in my own experience they are very rarely met with in the usual course of events.

EXPERIMENT IV

STRAIN OF ORGANISM	DILUTION OF POLYVALENT SERUM						
	1 TO 0	1 TO 5	1 TO 10	1 TO 15	1 TO 20	1 TO 25	1 TO 30
No. 1	0	0	0	0	0	0	0
No. 2	0	0	0	0	0	0	0
No. 3	0	0	0	0	0	0	0
No. 4	0	0	0	0	0	0	0
No. 5	0	0	0	0	0	0	0
No. 6	0	0	0	0	0	0	0
No. 7	0	0	0	0	0	0	0
No. 8	0	0	0	0	0	0	0
No. 9	0	0	0	0	0	0	0

This experiment is designed to determine the highest dilution of polyvalent antidysentery serum that will give nonspecific agglutination of strains of organisms that produce colonies simulating those of the dysentery group on the modified medium. *S* indicates slight agglutination; *O* indicates no agglutination. Time of observation extended over one hour.

As complete agglutination of all the strains of dysentery occurred in a dilution of 1-50 and as practically no false agglutination took place in the nondysenteries, a dilution of 1-30 was thought to be entirely safe both from the standpoint of sensitiveness and safety to employ as a routine procedure.

As the eosin-methylene blue agar, as it is adjusted for this work, is a very favorable medium for the E. dysentery, good results can be obtained at the end of 12 to 15 hours after the sample has been planted. This feature of the method is very desirable from the clinician's viewpoint, and the elimination of the usual broth transplant to obtain material for an agglutination is a simplification of the older methods of rapid identification.

Although it is admitted that there are several weak points in this method of identifying an organism so elusive as those of the dysentery group sometimes are, it is possible to check very closely with the more elaborate methods. In the hands of experienced workers it is thought that the errors due to fallacies in the technic will not be great enough to overcome the advantage of a rapid diagnosis.

As the local dysentery season was practically over by the time the details of this method of diagnosis were worked out, I am not prepared to give

the results of its practical trial in a large number of cases. However, in a limited number of samples of suspected dysentery cases excellent results were obtained. In no case where a negative result was obtained did the subsequent clinical evidence point to error. In only one case was a positive agglutination obtained that gave a negative dysentery culturally, and in that case the agglutination was probably the more reliable criterion.

SUMMARY

1. There is a need for a rapid and reliable bacteriologic identification of members of the dysentery group.

2. A method for such an identification is proposed, which has as its salient features a modified eosin-methylene blue agar that is more selective than the older medium and a slide agglutination for the identification of the suspected organisms that is time saving and is also reliable.

3. A diagnosis can be made by this method at the end of 12 to 15 hours which is very desirable, in that it affords a definite indication to the clinician in the shortest possible time.

4. Experimental evidence is given in support of the various phases of the method.

THE SPECIFICITY AND SENSITIVENESS OF THE AUTHOR'S NEW COMPLEMENT-FIXATION TEST FOR SYPHILIS*

By John A. Kolmer,† M.D., Philadelphia, Pa.

DOUBTLESS all will agree that the two most important questions concerning the complement fixation or any other serological test for syphilis are *specificity* and *sensitiveness*. All other considerations should be of secondary importance and especially simplicity and economy of time and materials. When one stops to consider that syphilis is a widespread disease of great economic importance involving men, women and children of all classes, responsible for a heavy infant mortality and a large percentage of chronic diseases of adults, and that in many cases the diagnosis is greatly aided and indeed, may be only possible by the complement-fixation test, the questions of accuracy and sensitiveness must be conceded as the prime factors of importance.

I believe it is the duty and one of the heaviest responsibilities of physicians practicing laboratory medicine and diagnosis, to employ a method ensuring the highest degree of reliability not only because the diagnosis of syphilis frequently depends largely and sometimes solely upon the complement-fixation test, but likewise because the treatment of this disease is usually

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controlled by the results of these reactions, and no one can deny that for this purpose the test should yield a reaction as sensitive as is consistent with specificity in order to avoid the regrettable error of undertreating the disease.

In my opinion the Wassermann reaction is one of the most valuable tests in medical science. Unfortunately, however, it is a complicated method for the inexperienced although perfectly simple for the trained and experienced immunologist. Largely as the result of its abuse in the hands of insufficiently trained workers inside and outside of the medical profession and especially as a result of numerous modifications aiming to evolve a shorter and simpler method, the true value of the complement-fixation reaction in syphilis is greatly underrated by many physicians. However, the practitioner cannot be blamed for this attitude since he is able to judge only by results, possesses little or no time for mastering the principles and simply fails to understand why positive reactions are observed with the sera of non-syphilitic persons and especially why different laboratories will return reports with portions of the same blood differing as widely as night from day. It is the duty of serologists to guard their work with the utmost care; to evolve and employ a method of the greatest technical accuracy and then to patiently and continually reeducate the practitioners of medicine on the interpretation and true value of the complement-fixation reaction. It ought not to be necessary to apologize or hedge on the results of our work and we ought not to employ a method in which we have no confidence in the weakly positive reactions. As I hope to point out later, serologists cannot hope to ever evolve a method capable of detecting all cases of syphilis; if the *Spirochete pallida* is capable of lying so dormant in the tissues that even the usual admiring circle of lymphocytes and plasma cells is absent, there is no hope of being able to detect in the test tube sufficient amounts of reagin when this is practically absent from the plasma. But in that large group of cases containing small amounts of reagin in the blood, the very group in which our complement-fixation test should be of most value, the reactions are apt to be weak and we should be just as sure of these as with more active cases of syphilis yielding stronger reactions.

There is not any more merit in securing positive reactions in active cases of syphilis than in making an x-ray picture of a long bone that can be bent double, for a fracture; our aim should be to provide the practitioner with a method for the accurate diagnosis of syphilis when such is not possible or doubtful by clinical means.

Six years ago my associates and I set out to determine more accurately the sources of error in the Wassermann reaction, hoping that a minute study of every step and phase of the test would yield such information as to enable us to gradually build up a technic of acceptable accuracy and sensitiveness. To know a source of error intimately, its nature and mechanism, usually permits one to adopt adequate and acceptable means for preventing its occurrence and we hoped to attain accurate information of this kind on all the important and minor steps and phases of the complement-fixation test in syphilis. After completing a study of this kind a new method was built up[1]

which we have found much superior to both the original Wassermann reaction and a modification of it employing three antigens, which I described in 1915 and which has since come into widespread use. We have not called this new method a standardized test as this designation must be earned and granted by the consent of the majority but we have found it a method yielding specific and very sensitive reactions and trust that it at least merits a fair and sufficiently extensive trial alongside other methods.

THE SPECIFICITY OF THE NEW COMPLEMENT-FIXATION TEST

Probably the most important single factor of the complement-fixation test for syphilis is specificity. The damage that may be done by a falsely positive reaction is sometimes tremendous and especially if it falls into the hands of a physician who accepts the results without question. In order to avoid the possibility of this error most serologists have purposely adopted a method known to be below the average in sensitiveness, being willing to "miss" many cases of syphilis in order to avoid the risk of nonspecific reactions.

As I have already discussed in a former article,[2] falsely positive reactions may be due to technical errors or to biological phenomena. The former is the more usual and important in this country at least; the latter occurs only in frambesia or yaws, a tropical disease produced by *Spirocheta pertenuis* and capable of yielding true positive Wassermann reactions with technically perfect methods.

Technical errors are, or should be, capable of detection and correction; the possibility of biological errors resolves itself into the question of whether or not the Wassermann antibody or reagin is produced in any diseases other than syphilis and frambesia.

A review of the literature and especially the early literature on the Wassermann reaction shows that positive reactions have been recorded by some one at some time in practically all diseases of human beings. We now know, however, that much of this work has been based upon technical errors, although in a survey of any group it is entirely likely and to be expected that some cases of latent and unsuspected syphilis will be encountered yielding true positive reactions but regarded as a nonspecific or falsely positive reaction due to the disease with which the individual may be suffering in addition to syphilis.

I do not deny that in leprosy, diabetes, uremia, jaundice, etc., such profound changes may occur in the colloidal chemistry of the plasma that the chances of securing nonspecific reactions are greatly increased but experience with my new complement-fixation test has convinced me that these may be avoided by technical measures.

During the past two years I have had the good fortune of securing the cooperation of Dr. Oswald E. Denney of the Public Health Service and surgeon in charge of the leprosarium at Carville, Louisiana, in a study of the new test in leprosy; of Dr. Lewis B. Bates, director of the Board of Health laboratory of the Panama Canal zone; and Dr. R. L. Cook, senior surgeon in

charge of the U. S. Veteran's Hospital No. 25 in Houston, Texas, in a study of the new test in malaria; of Dr. Edward Steinfield, assistant professor of medicine in the Graduate School of Medicine of the University of Pennsylvania, in a study of the new test in diabetes, uremia, jaundice, etc., and of Dr. Frederick C. Smith, graduate student in gynecology and obstetrics in the University of Pennsylvania, in a study of the sera of pregnant women.

Dr. Denney and I have published the results of our study in leprosy elsewhere[3] and found that in nonsyphilitic lepers the new test yielded uniformly negative reactions, although the old test yielded apparently falsely positive reactions with 7.2 per cent of serums. Unquestionably in some cases of leprosy there is an increased tendency of the serum to yield falsely positive reactions and especially in tests employing alcoholic extracts of tissue saturated with cholesterol. But in my new test this tendency is neutralized by the use of a new antigen largely free from anticomplementary activity and by other technical improvements. Dr. Denney believes that a positive reaction with the new method justifies the clinician in proceeding with antisyphilitic treatment without the mental reservation that he may be uselessly subjecting a leper to unnecessary or even harmful measures.

Dr. Smith has likewise reported his work elsewhere[4] but here it may be stated that tests conducted with the sera of 94 women in the last few weeks of pregnancy yielded consistently negative reactions with all except five and all of these continued to react positively after delivery and were regarded as syphilitic.

The study in malaria has not yet been completed but up to the present time the serums of 25 cases have been studied with the results shown in Table I. In the first place it may be mentioned that of 27 sera sent to Philadelphia from Ancon and Houston, only two proved anticomplementary in the new method. Furthermore all of a series of bloods from syphilitics sent me by Dr. Bates from the Canal Zone, yielded positive reactions showing that there was no loss of fixing power of the serums. The majority of these serums were from estivo-autumnal cases; five were from tertian infections. Blood was drawn from a few cases during a chill; in most, however, the specimens were secured from a few hours to three days after paroxysms. All except two yielded negative reactions. Both of the positive reacting sera were derived from Negro men two to three days after paroxysms; one of these men subsequently died and Dr. Bates was unable to discover macroscopic evidences of syphilis in his aorta and other organs although he states in a letter that this does not rule out the possibility of syphilis and that during 1923 twelve of his autopsy cases which had yielded positive Wassermann reactions in his laboratory during life showed no gross evidence of syphilis in the tissues. Since Warthin's epoch-making studies in the pathology of syphilis[5] we know that syphilis cannot be excluded by macroscopic examinations alone of organs at the necropsy table.

Dr. Steinfield and I are preparing to publish a detailed report on the new test conducted with sera from cases of diabetes mellitus showing from

TABLE I

RESULTS OF NEW COMPLEMENT-FIXATION TEST IN NONSYPHILITIC DISEASES

CLINICAL	TOTAL EXAMINED	POSITIVE	NEGATIVE
Syphilitic lepers	27	27	
Nonsyphilitic lepers	125	None	125
Lepers; syphilis suspected	6	None	6
Pregnant women	94	5	89
Diabetes mellitus	32	None	32
Jaundice	14	None	14
Uremia; chronic nephritis	15	None	15
Pneumonia (Lobar)	15	1	14
Malaria	25	2	23
Pernicious anemia	2	None	2

0.150 to 0.400 gm. and more sugar per 100 c.c. of blood and many in diabetic coma; all of a series of 32 cases yielded negative reactions. Likewise all of a series of 15 nephritics with marked retention of urea and many in uremia, yielded negative reactions. In a series of 15 cases of croupous pneumonia embracing all of the serologic types, the reactions were negative with all except the serum of one syphilitic. All of the sera of 14 individuals with severe jaundice due to gall-bladder and liver disease and carcinoma of the pancreas, yielded negative reactions as likewise the sera of two cases of pernicious anemia.

In an extensive study of the tuberculosis complement-fixation test by Dr. Andrew Callahan, assistant professor of medicine in the Graduate School of Medicine of the University, and myself, to be published later, the new test for syphilis was applied to all with uniformly negative results except with the sera of those known or strongly suspected as being syphilitic as well as tuberculous.

In addition to these I have conducted tests with the sera of a number of cases of acute and convalescent scarlet fever with uniformly negative results and I believe on the basis of these experiences that the new complement-fixation test does not yield falsely positive reactions when the method is applied in a technically correct manner. It cannot be hoped that any method including mine is free of the possibility of technical error; I am prepared to learn of apparent nonspecific reactions in the hands of others and myself, but I firmly believe, that as experience is gained with the method, others will agree with my associates and myself that technical errors are reduced to a minimum and that in this country, at least, where yaws is practically unknown, the positive reactions may be accepted as evidence of syphilis.

Furthermore, it cannot be hoped or expected that clinical opinions will always agree with the results of serologic reactions in either a positive or negative way. The clinician may be just as sure of his diagnosis as the serologist is of his, but I do believe that when this new test yields a positive reaction that the burden of proof is on the clinician. Time and time again I have found this to be the case and usually that the physicians have been misled by negative histories until patients have admitted venereal sores under the pressure of positive and repeatedly positive reactions.

Finally, it should not be overlooked that in latent syphilis yielding negative complement-fixation reactions, trauma, pregnancy, intercurrent infections and various types of tissue injury may stimulate the spirochetes into renewed activity with the production of true positive reactions which may be but temporary and analogous to the so-called provocative Wassermann reactions sometimes following the administration of arsphenamine and neoarsphenamine. Such reactions are easily regarded as nonspecific but are truly positive and the possibility of their occurrence under these conditions should not be overlooked.

THE SENSITIVENESS OF THE NEW COMPLEMENT-FIXATION TEST

As previously stated the complement-fixation test for syphilis should be as sensitive as is consistent with specificity. In the diagnosis of the chancre this is not of prime importance because laboratory diagnosis is better aided by dark field microscopic examination of the secretions for *Spirochete pallida;* neither in the secondary stage of syphilis when the diagnosis is usually readily made alone by clinical examination. But in the tertiary and especially in the latent stages of the disease when the clinical diagnosis is doubtful or indeed impossible, and the serologic diagnosis of most value, the complement-fixation test should be as sensitive as is possible with specificity. This is especially true for the detection of latent syphilis in women and in congenital syphilis of both children and adults in which clinical diagnosis is frequently impossible. Furthermore when the treatment of syphilis is being guided by Wassermann reactions, the method should be as sensitive as possible with specificity. Most of us are not overtreating syphilis; rather the tendency is to undertreat the disease and I believe that a specifically sensitive complement-fixation reaction with both blood serum and spinal fluid constitutes one of the most important guides in the treatment of syphilis and insures more than any other single procedure the adequate treatment of the disease.

As previously stated we cannot hope to detect all cases of syphilis by the complement-fixation or any other test or examination known today. The value of the history is sharply limited; all physicians treating syphilis know that not only may some patients persistently and untruthfully deny venereal infection, but that some are perfectly sincere and truthful in denying all knowledge of the disease. And this is readily understood and especially in cases of women, when it is remembered that the primary lesion may be so small or so situated as to escape detection not only by the patient but by the physician as well.

Furthermore, the clinical manifestations of tertiary syphilis may be so atypical and misleading as not to lead even to a suspicion of syphilis unless a complement-fixation reaction is conducted. And in the latent stages of the disease when the patient is apparently in good health or suffering with some additional nonsyphilitic disease, the physician may have absolutely no grounds or reason for suspecting luetic infection. I repeat, therefore, that not infrequently the physician cannot say that syphilis is or is not present in such cases and I am absolutely sure that in not a few the complement-fixation test is the only, or at least the main and best, means for diagnosis.

This does not mean that a positive complement-fixation reaction always indicates that a particular lesion is syphilitic; physicians of experience have frequently seen the tuberculous ulcer of the larynx regarded as syphilitic because the patient was luetic and yielded a positive reaction. We must not forget that the syphilitic is susceptible to tuberculosis, cancer and other diseases. Altogether too frequently we permit the diagnosis of syphilis to shut our eyes to the possibility of other diseases in the sick individual; we all know that it is indeed difficult for the luetic to recover from the "mental scars" of his disease but the physician especially should avoid falling into the same error of ascribing every ache and pain of the syphilitic to syphilitic infection.

My purpose in building up the new complement-fixation test was to render it as sensitive as possible with specificity. To attain these ends a new antigen was discovered and numerous technical details included and no effort was made to popularize the test by turning out a short cut method. I am convinced that enough mischief has been done by such methods; let us realize and admit that the complement-fixation test is intricate and that it requires skill and experience as in most other lines of work and endeavor in medicine. Let us admit that a mere additional twenty-four hours and a little more labor are as nothing compared with accuracy. Why and how a physician will insist and demand a report on the same day that he sends a specimen of blood or spinal fluid to the laboratory is beyond my comprehension when the question of accuracy is involved. I can readily enough understand how a day makes some difference in primary syphilis when treatment should be instituted as promptly as possible, but here we should not rely upon the complement-fixation test but proceed with the dark-field examination which in experienced hands yields its results in a matter of thirty minutes. But even if the complement-fixation test is required, an additional wait of twenty-four hours makes little difference. But in chronic syphilis when months and even years have elapsed, insistence upon an immediate or almost immediate report is almost foolish even though the patient may be retained in a hospital for an extra day or two.

My new method, therefore, does not aim to be a short cut method and a serum received in the morning cannot be reported upon until the following morning because of the 18 hour primary incubation in the refrigerator. We could not escape adopting this method; an enormous number of comparative tests proved conclusively that the ice-box method yielded the most sensitive reactions which were at the same time specific and in our search for the best this fact could not be overlooked or ignored even if it meant increasing the sensitiveness of the reaction by less than 10 per cent.

In addition to employing a new antigen and refrigerator primary incubation, numerous other technical details have been introduced, some of which tend to increase slightly the time and labor required for conducting the test, but all of which I believe add their mite toward increasing specificity and sensitiveness. In our experience the new method has proved equal in sensitiveness to tests employing raw or unheated serum without danger of non-

specific reactions. I believe the new test serves for the detection of smaller amounts of antibody or reagin in the blood and spinal fluid of syphilis than most other methods in common use as well as yielding a quantitative reaction of particular value in guiding and evaluating the treatment of syphilis. In the primary stage of the disease positive reactions are obtained several days earlier than by other methods and likewise in the secondary, latent and tertiary stages the percentage of positive reactions is much higher and more specific than obtained by the usual methods as found not only in our work but likewise by Shivers,[6] Schamberg and Klauder,[7] Kilduffe,[8] Palmer and Gibbs,[9] Harper and Curtis,[10] Schamberg and Greenbaum,[11] and numerous others who have been kind enough to furnish me with reports of their work and experience with the method.

I know that many of the methods employed at present are excellent in every particular and particularly those used by serologists of experience; I do not expect and cannot hope that the new method will prove superior to these, but I believe it will prove equal in specificity and sensitiveness and if a standardized method is advisable, as stated by several writers, in order to have a uniform method for the reduction of confusion and to provide a means for comparing and evaluating different methods for the treatment of syphilis, I hope that the method will be given a sympathetic, fair and extensive trial. In our experience together with that of the investigators just mentioned, nonspecific reactions have not occurred in a total of over 15,000 tests and an increased sensitiveness has been commonly noted. Some quality of the new test and probably the new antigen, which permits the use of a dose of 10 units which is at least 30 to 50 times less than the anticomplementary unit, has imported to it an extremely high degree of specificity associated with sensitiveness, and it is earnestly hoped that the fulfillment of these two qualities of first importance will outweigh the fact that the method requires a little more skill and time than most methods in use at present.

BIBLIOGRAPHY

[1]Amer. Jour. Syph., 1922, vi, 469, Series of thirty-two papers in Amer. Jour. Syphilis beginning, 1919, iii, 1.
[2]Amer. Jour. Med. Sci., 1923, clxv, 320.
[3]Arch. Dermat. and Syph., 1923, viii, 63.
[4]Amer. Jour. Syph., 1922, vi, 705.
[5]Amer. Jour. Syph., 1918, ii, 425.
[6]Arch. Dermat. and Syph., 1922, vi, 344.
[7]Medical Clinics of North America, W. B. Saunders Company, Phila., 1921, 667.
[8]Arch. Dermat. and Syph., 1922, vi, 709.
[9]Arch. Dermat. and Syph., 1922, vi, 738.
[10]United States Naval Medical Bulletin, 1922, xvii, 757.
[11]Jour. Amer. Med. Assn., 1923, lxxx, 836.

(*For discussion see p.* 493.)

COMPARATIVE RESULTS ON FOURTEEN HUNDRED TESTS IN WHICH BOTH THE KOLMER METHOD AND ROUTINE WASSERMANN TECHNIC WERE USED*

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THIS report shows the comparative results obtained in a series of fourteen hundred Wassermann tests in which both Kolmer's method and our routine technic were used. The Kolmer tests were begun in September, 1922, by Cohen, with reagents furnished by Kolmer for the purpose of comparative observations in as large a series as possible. The work was done at the Elizabeth Steel Magee Hospital Laboratory and the specimens were submitted chiefly by the house and out-patient services of that hospital, by the venereal clinic of the Pittsburgh Eye and Ear Hospital and by the Tuberculosis League. Most of the specimens were sera though a fair number were spinal fluids. Practically all of the specimens were accompanied by histories, a printed outline having been furnished to the clinicians for the purpose.

Kolmer's technic was adhered to, strictly in accordance with his instructions[1] and while the antigen furnished by him gave out, we prepared new antigen by his method[2] feeling that the ability of the individual laboratory to prepare a reliable antigen according to directions enhanced, rather than deducted from, the value of the experiment.

Our "routine" technic is a modification of the original Wassermann sheep system, and as used at present is a refinement of a method installed by C. C. Hartman at the Laboratory of the Allegheny General Hospital in 1913. It was later adopted at the Singer Memorial Research Laboratory, Pittsburgh, where slight improvements and minor additions were made from time to time by Haythorn, Allison, and Lacy. In a general way, we have had ten years' experience with this technic and it has been in use practically without change for the last six years.

TECHNIC OF THE "ROUTINE" TEST

All sera are cleared by centrifugalization and the resulting clear fluids inactivated by heating at 56° C. for twenty minutes; washed sheep cells are then added and the natural antisheep amboceptor absorbed out. The sera are then ready for testing and constant quantities of 0.2 c.c. are used in each tube of the test. Two antigens are always used, a cholesterin saturated alcoholic extract of human heart and a pure lipoidal acetone insoluble fraction of an alcoholic extract of beef heart. Each is diluted so as to contain

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at least four units as titrated against pooled known positive sera. The amboceptor and complement are titrated on each day of use and the latter is used in 1:20 dilution. A 2.5 per cent suspension of washed sheep cells is used in the hemolytic system and a fairly constant strength is assured by accurately measuring the red cells from a centrifuged sample obtained by running the centrifuge for exactly five minutes, at a given speed which is regulated by always placing the lever on the same notch of the controlling rheostat.

Four tubes are used in every test and all of the possible reagent combinations are controlled in addition. Cholesterinized antigen is placed in the first tube, lipoidal antigen in the second tube and patient's serum and complement are then placed in each of the four tubes. After one hour's incubation at 37° C. sensitized cells are added to each of the first three tubes, plain unsensitized cells to the fourth tube and all are again incubated for an hour. The tests are then read, and placed in the refrigerator overnight. In the morning a second reading is made, since a much better idea of the percentage of hemolysis can be arrived at in this way. The first tube shows the amount of fixation of the cholesterinized antigen, the second, the amount of lipoidal antigen fixed, the third, the anticomplementary properties, if present and the fourth, whether or not any natural antisheep amboceptor has remained after the absorption process.

Some idea of the reliability of the "routine" method is given by an analysis of the results of 7074 tests made with it at the W. H. Singer Memorial Research Laboratory which was reported by Haythorn[3] in 1921. It was found that 82.9 per cent, or 5802 of the tests were negative; 11.3 per cent, or 794, were four-plus positives and gave complete fixation with both antigens; 5.8 per cent, or 404, were partial positives, and gave partial fixation with one or both antigens; and that one per cent, or 74, tests were anticomplementary.

Further analysis in the light of the information furnished by the histories indicated that the greater number of partially positive tests occurred in treated syphilis, in latent syphilis in which the symptoms appeared years after the primary lesion, or were obtained upon the blood sera of patients having cerebrospinal syphilis.

False positives were obtained during the febrile stages in three cases of pneumonia, two of typhoid fever and one each of chorea and acute arthritis, and in eight pregnant women, just prior to term.

COMPARISON OF RESULTS

In the beginning it was decided to continue to use the routine method on all specimens submitted and to parallel the test with the Kolmer technic in every instance where the sample was large enough to complete both tests. It was further decided to keep up the data and to analyze them as we went along, paying special attention to those conditions in which the routine method had been found to vary, or to disagree with the clinical findings, as shown by the former analysis. Therefore, we concentrated our attention on treated cases, pregnancy, febrile diseases and false positives of all types.

It was an easy matter to determine the total number of positive and

negative tests by both methods but was difficult to reach a satisfactory basis for making comparative readings for the partially positive or weakly positive ones, since the number of tubes and the strengths of the red cell suspensions, in the two technics, differed fundamentally. Before tabulating the results the tests were separated into negative tests by both methods, positive tests by both methods, partially positive tests by both methods, disagreements, and anticomplementary tests. Definitions of these terms as they concern the relations of the two technics are necessary to a complete understanding of the tables.

1. Negative Tests. Tests were considered negative in both methods when there was complete hemolysis in all of the antigen-containing tubes, provided the control tubes were satisfactory.

2. Positive Tests. Complete inhibition of hemolysis in both tubes or complete inhibition with the cholesterinized antigen and more than 75 per cent inhibition with the lipoidal antigen as estimated on the following morning in the routine method was taken as an unqualified positive test, while complete inhibition in the first three or more tubes in the Kolmer set was taken as the comparative standard of an unqualified positive test.

3. Partially Positive Tests. Partially positive tests included those in which there was from 25 per cent to 100 per cent inhibition with the cholesterinized antigen and from less than 25 per cent to 100 per cent hemolysis with the lipoidal antigen by the routine method, and those in which there was definite fixation, in the first two or more tubes of the Kolmer set and less than complete fixation in any of the first three.

4. Disagreements. Tests which proved to be negative with one system and either positive or partially positive with the other were classed as disagreements.

5. Anticomplementary Tests. All tests in which hemolysis in the anticomplementary control tubes was incomplete were classed as anticomplementary.

When the number of Kolmer tests reached fourteen hundred, the calculations were begun so that the percentages could be figured in round numbers and the following Table of gross results obtained.

TABLE I

Tests, negative by both methods	1019 or	72.7	per cent
Tests, positive by both methods	288 or	20.57	" "
Tests, partially positive by both methods (variation in degree)	23 or	1.6	" "
Disagreements, positive by one test—negative by the other	55 or	3.9	" "
Result, anticomplementary by routine method only	4 or	.29	" "
Result, anticomplementary by Kolmer method only	10 or	.72	" "
Result, anticomplementary by both methods	1 or	.07	" "

The high percentage of positives is explainable on the nature of the clinical material, largely obstetrical and venereal, and on the fact that many tests were from patients under antisyphilitic treatment.

A further analysis of the partially positive tests shows that most of them occurred in treated syphilis, and that the variations were rather in degrees of fixation than in actual contradiction. Of the 22 tests which were partially

positive by the routine method and either partially positive, or strongly positive by the Kolmer method, 17 samples were from patients under treatment for lues. (See Table II.)

TABLE II

PARTIAL POSITIVES

Positive with routine test:	Partial Positive with Kolmer test	1
Partial positive with both tests		8
Partial positive with routine test:	Positive with Kolmer test	14
Total		23

A careful study of the tests which were actually contradictory shows them to be almost nil. Only one test was strongly positive by the routine and negative by the Kolmer method and when repeated at a later date was strongly positive with both methods. Of the 33 partial positives (Table III) with the routine method which were negative with the Kolmer test, 19 were from patients in the later months of pregnancy and only one of the latter showed any fixation of the lipoidal antigen. Of the 20 tests which were negative by the routine method and either partially, or strongly positive by the Kolmer test, 12 were from patients who were receiving antispecific treatment. Table III shows the relative number of disagreements.

TABLE III

DISAGREEMENTS

Positive routine test:	Negative Kolmer test	1
Partially positive routine test:	Negative Kolmer test	33
Negative routine test:	Partially positive Kolmer test	13
Negative routine test:	Positive Kolmer test	7
Total		54

The anticomplementary tests were very interesting and are set forth in Table IV.

TABLE IV

ANTICOMPLEMENTARY TESTS

Routine test, anticomplementary:	Kolmer test, negative	3
Routine test, anticomplementary:	Kolmer test, positive	1
Routine test, negative:	Kolmer test, anticomplementary	5
Routine test, positive:	Kolmer test, anticomplementary	5
Anticomplementary by both methods		1
Total		15

About two years ago we had the experience of seeing the number of anticomplementary tests coming from one of the clinics reduced from over 10 per cent to less than one per cent by the adoption of a rigid technic which insisted upon the sterile collection of all blood samples in clean, dry, water and chemical free containers and the immediate transportation of the specimens to the laboratory. We have felt, therefore, that anticomplementary results could be eliminated if such technic was generally enforced. The varying results obtained by the two technics indicate, at least in all but one of the tests, that either the technic was responsible or else the disturbing factor was inherent in the serum itself. At any rate, the often heard assertion that

anticomplementary tests are generally imperfect positives is apparently erroneous, since there were as many anticomplementary Kolmer tests which turned out negative with the routine method as there were positives, while the majority of the routine anticomplementary tests proved to be negative when controlled by the Kolmer test.

Viewed from the standpoint of satisfactory results the comparative tests were divided into those with results in treated cases, results in pregnancy and results in miscellaneous conditions.

The Kolmer test gave the larger number of positive tests in treated syphilis. During the treatment of a patient who has had a strongly positive Wassermann, the amount of fixation with the lipoidal antigen usually begins to decrease gradually, and after repeated tests disappears altogether, while the cholesterinized antigen persists for a long time. Kolmer's test was found to persist for a still longer time than the latter. In 12 specimens it remained strongly positive even after inhibition with the routine cholesterinized antigen had disappeared entirely, and in many other instances it persisted as a weakly positive test. In three exceptional instances, the fixation of the routine cholesterinized antigen persisted for a longer time than did the Kolmer test.

In the late months of pregnancy the Kolmer test apparently had the advantage of the routine method. Only once was the Kolmer test strongly positive in pregnancy where the indications were against syphilis; though it was also negative in a case of known syphilis, positive by the routine test. The routine test likewise was positive, with both antigens in only one case of pregnancy in which syphilis was ruled out, but there were 19 specimens from pregnant women, probably not syphilitic, in which there was more or less fixation with the routine cholesterinized antigen.

The disagreements which occurred in miscellaneous conditions almost balanced each other. Kolmer's test gave a false positive in a child with suspected meningitis which was negative with the routine, and in which the parents of the child were negative with both tests. The Kolmer test was negative in several instances of apparently clinical syphilis in which the routine test was partially positive. False partially positive routine tests (cholesterinized antigen fixation) were obtained in two cases of malnutrition in children, in a patient with a large fibroid tumor and in one pneumonia case during the febrile stage. All of these latter sera were negative with Kolmer's method.

SUMMARY

In a series of 1400 Wassermann tests there was only 3.9 per cent in which the Kolmer test actually disagreed with the routine method in use. There were, however, many instances of variation in the degrees of strength of fixation and these were most commonly seen in treated luetic cases, where Kolmer's test remained positive after the routine test had become negative. In pregnancy, the Kolmer test gave fewer false positives than did the cholesterinized antigen of the routine test though one false positive result, in pregnancy, was obtained with it. We consider the Kolmer modification of the

Wassermann test an excellent and reliable method. However, it requires more time, more tubes, and more patience to carry through than the routine method does and in general, it appears to be open to most of the same errors. We have decided to continue the practice of both methods for the time being and to reserve our conclusions until more data is at hand.

BIBLIOGRAPHY

[1]Kolmer, John A.: Studies in the Standardization of the Wassermann Reaction. XXX A New Complement-Fixation Test for Syphilis Based upon the Results of Studies in the Standardization of Technic. Am. Jour. Syph., 1922, vi, 82.

[2]Kolmer, John A.: Studies in the Standardization of the Wassermann Reaction, XXV. A Superior Antigen for Complement-Fixation Tests in Syphilis (A Cholesterolized and Lecithinized Alcoholic Extract of Heart Muscle). Am. Jour. Syph., 1922, vi, 74.

[3]Haythorn, Samuel R.: The Practitioner's Part in Acquiring the Knowledge Necessary for the Intelligent Interpretation of Atypical Wassermann Reactions. Penna. Med. Jour. 1922, xxv, 602.

(*For discussion see p.* 493.)

A STUDY OF THE KOLMER METHOD OF COMPLEMENT FIXATION FOR THE DIAGNOSIS OF SYPHILIS*

By W. H. Kellogg, M.D., Amy Wells, and Dorothy Beck,† Berkeley, Calif.

CONFLICTING Wassermann reports from different laboratories are a source of confusion and the cause not only of doubt as to the value of the test but a mistrust of the laboratories, in the minds of clinicians. It is therefore to the interest of the laboratories that such discrepancies be guarded against in every way possible. The universal adoption of any one method, however imperfect it might be, would go far toward meeting the difficulty of conflicting reports, but the desirability of accuracy comes first, and any test to be considered at all, must therefore meet this requirement. The need for standardization of the serological diagnosis of syphilis is urgent and the utmost credit is due Dr. Kolmer for taking the lead, by perfecting, after a long and painstaking investigation, a technic which he now offers as being both safe and sensitive and worthy of general adoption.

It is quite likely, granting all the claims put forward for the Kolmer test that it has no monopoly of desirable characteristics and it is even possible that there may be other methods simpler in technic and possessing all the advantages of that test. Although we had become convinced after running several hundred Kolmer tests, that it was all it was claimed to be, still it seemed desirable to carry the matter further, especially since the inferiority of the test we were using as compared with Kolmer's was not yet demonstrated to our satisfaction. There were also certain features of Kolmer's test on which it seemed desirable to have more light; such as the reason for the scarcity of anticomplementary results, the rôle of the antigen, the greater number of one plus reactions, etc. Accordingly a new series of

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investigations was started, the results of which form the subject of this paper.

The plan adopted in this investigation was to run each one of a series of blood specimens by four different tests; first by Kolmer's method, the second by Kolmer's method with Noguchi's acetone insoluble antigen substituted for Kolmer's, the third by our own method, which will hereafter for convenience be called the State method, and the fourth test by the State method using the Kolmer antigen. To one serologist were assigned the two Kolmer tests and to another the two State tests, the same reagents being used in all tests.

It will be unnecessary to describe Kolmer's test but the State method is briefly as follows: An antisheep hemolytic system is used and the complement titrated daily but not the amboceptor. Noguchi's acetone insoluble antigen is used in the quantity of 5 units and the primary fixation is in the ice box for 16 to 18 hours. Two antigen tubes are used, one containing .08 and the other .04 c.c. of serum. Sheep's blood is used in the same quantity as the Kolmer test, but the total volume of the test is only 1 c.c. The amboceptor is standardized by titrating it against .2 c.c. of 1-10 fresh guinea pig complement and it is not retested except at long intervals. In the titration of complement and for use in the test, the dose of cells is sensitized with three units of amboceptor. This results in a smaller complement unit than would be the case if one unit of amboceptor is used. The complement which is pooled from several animals is titrated in the presence of antigen and used in the quantity of two units. Serums are inactivated for 15 minutes only, at 56° C.

The series examined consisted of 506 specimens taken consecutively as they arrived at the State Hygienic Laboratory, all accompanied by the official information form, filled out in varying degree of completeness. It may be objected that such a series is not suitable for an investigation of this kind on account of the lack of reliable clinical knowledge concerning the cases as well as doubt concerning the diagnostic ability of the senders. There are a number of reasons for our belief that in the present instance these are not valid objections. In the first place we have a fairly large series, 506. In the second place a large percentage of our work is from clinics and institutions in charge of men whose clinical experience is large and who have become accustomed to our insistence on information. Thirdly, a few points selected from a general summary of results are significant, such as the following: Of those serums without a history and therefore principally routine and not diagnostic examinations, 378 were negative in all tests, 11 showed a one-plus in only one test and only 48 were definitely positive. Of those giving a definite or suspected diagnosis of syphilis or a record of antisyphilitic treatment, 59 were positive and only 24 negative. It may, I think, be very reasonably assumed that most of the serums that were negative in all four tests were from nonsyphilitic persons, and that most serums positive in more than one test were from syphilitics. It was therefore considered that a very fair approximation for a division of the serums into two groups, one of them probably syphilitic and the other probably not, would be to

place under the first group all cases giving a definite clinical history and also all cases giving a positive result in two or more of the four tests when no history was given. The second group is made up of all cases without a clinical history and that are negative in all four tests, those that have a doubtful history and are negative in all four tests and those that have no history and show only one-plus in only one of the four tests.

The Kolmer method used was the one tube, so-called qualitative test, as it does not seem likely that the quantitative method will ever be very generally adopted as a standard routine test. In our previous comparative tests, the importance of taking into account the differences in detail of various methods and of arranging to make conditions strictly comparable was demonstrated and the ideas gained were applied to the series herewith reported. To secure uniformity and consequently just comparisons, the State tests were read by Kolmer's method; i.e., the tube containing the most serum was taken and after reaction was complete, sufficient normal salt solution was added to make the bulk the same as in the Kolmer tubes. The Kolmer reading scale was used and all tubes centrifugalized before reading. The tubes containing the smaller amount of serum were disregarded, although we are of the opinion that a two tube test gives closer readings than one tube even with the reading scale of Kolmer. For example, out of 336 tests in which both methods of reading were used, there were 14 which were recorded as 4-plus by Kolmer and 4-plus by the State when read by Kolmer's scale, which is applied only to one tube, but which were really 3-plus when read by the State two tube method; the latter records as 3-plus any test with complete inhibition in the tube containing the most serum and partial inhibition in the tube containing the least amount of serum.

There were also two tests that would have been recorded as stronger by the two tube reading and properly so as they belonged to that small percentage of serums giving stronger reactions in smaller amounts of serum; a phenomenon that has been observed by many and is mentioned by Kolmer. As a further insurance of uniformity, the hemolysin and complement were the same in all tests but titrated especially for each test according to the method peculiar to that test. For example, the complement for the State test was titrated according to our method and in the same bulk used in that test and in the presence of the particular antigen to be used. Likewise, each of the two antigens was standardized for each of the two tests in which they were to be used.

Proceeding then to an analysis of the results obtained, we find as previously stated that the division of cases gave us 118 probably syphilitic and 388 nonsyphilitic, a proportion that has the appearance of being approximately correct. This segregation is probably as close to the actual facts as can be reached by any method, even that of limiting consideration to cases actually referred with complete clinical but not serologic history; for it must be admitted that purely clinical evidence is fallible and it must also be admitted that a properly performed Wassermann test has a very high degree of dependability on the positive side.

Of the 118 serums from syphilitics, treated and untreated, 28 were the same in all tests and may therefore be eliminated as of no further interest because no conflict was found. This leaves a total of 90 serums to be studied. A comparison of the results of these 90 tests all made four times, twice with Kolmer's method using two antigens and twice with the State method using the same two antigens brings out very strikingly one point; and that is, the variation in reaction of a given syphilitic serum to different antigens, some being positive with a cholesterinized antigen and weaker or negative in the acetone insoluble, and vice versa. Tables I and II illustrate this. In the first table all serums in the group of 90, that showed stronger reactions in the two tests with cholesterinized antigen are grouped together, while in Table II all serums showing the opposite are grouped. The evidence is plain that antigen more than anything else is the determining factor in differences in this group and no one antigen is superior to others for all cases.

TABLE I

KOLMER TEST	KOLMER TEST WITH NOGUCHI ANT.	STATE TEST WITH KOLMER ANT.	STATE TEST WITH NOGUCHI ANT.
++	−	++	+
+	−	+	−
++++	−	++	−
+	−	+	−
+	−	+	−
+	−	+	−
++	−	+	−
+++	−	++	−
+	−	+	−
+	−	+	−
++	−	+++	−
++++	++	++++	++
++++	−	+++	−
+++	−	++	−
+	−	+	−
+++	+	++	+
+	−	+	−

TABLE II

KOLMER TEST	KOLMER TEST WITH NOGUCHI ANT.	STATE TEST WITH KOLMER ANT.	STATE TEST WITH NOGUCHI ANT.
+++	++++	+	++++
+	++++	−	++++
−	+++	+	++++
+	++++	+	++++
+	++++	−	+++
+++	++++	+++	++++
−	++	+	+++
+	++++	+	++++
−	+	−	+

That there is some difference ascribable to other factors than antigen is shown by a grouping of tests according to method. In one group those that are better by the Kolmer method regardless of antigen, and the other group, those that are better in the State method. The groups are about equal, 30

in the Kolmer and 34 in the State. The reactions average stronger, however, in the State test.

In the entire series of 506 tests, two serums were anticomplementary in the State with Noguchi antigen, three in the State test with Kolmer antigen and none in the Kolmer's test, with either antigen. The difference here is plainly one of dilution as the bulk of Kolmer's test is 3 c.c. and of the State test 1 c.c. This was proved by retesting the same serums by the State method, unchanged except by increasing the bulk to 3 c.c.

In the entire series of 506 tests there were 10 serums showing a one-plus in one test only, no history being given. All of these were with cholesterinized antigen, six with Kolmer's test and four with the State test using Kolmer's antigen. These at least show a greater sensitiveness of cholesterinized antigen to slight influences, either small amounts of antibody or nonspecific complement fixation. We are inclined to believe with Kolmer that they are probably correct, when an antigen similar to that used by him is employed.

The acetone insoluble antigen gives more 4-plus positives in both tests than the cholesterinized antigen but the latter gives more 1-, 2- and 3-plus reactions, and fewer negatives.

TABLE III

	A		B	
DEGREE OF REACTION	KOLMER TEST	KOLMER TEST WITH NOGUCHI ANT	STATE TEST WITH KOLMER ANT	STATE TEST WITH NOGUCHI ANT
–	**10**	32	**11**	20
+	**19**	13	**24**	14
++	**15**	8	**12**	5
+++	**20**	9	**25**	13
++++	26	**28**	15	**36**
Anticomp.	0	0	3	2

Table III shows this peculiarity graphically. When comparing one test with another using a different antigen, the heavy faced type accentuates the preponderance in number of reactions on one side or the other.

SUMMARY AND CONCLUSIONS

1. The Kolmer test utilizes a particularly favorable adjustment of the various factors entering into the complement-fixation test for syphilis, and is, we believe, as sensitive as any of the recognized methods using inactivated serum and more sensitive than most others.

2. The delicacy of the Kolmer test is due to the antigen used, and to the adjustment between the dose of blood cells, the complement unit and the total volume of the test.

3. Comparative tests with other methods show that the refinements of technic which include the manner of titrating reagents and of reading results are not necessary to achieve the same results.

4. Noguchi's acetone insoluble antigen gives stronger reactions in definitely positive serums than the cholesterinized antigen of Kolmer, but it misses a few of the one-plus reactions.

5. We favor the adoption of Kolmer's test as a standard of comparison

if not for general adoption. The wide publicity and numerous favorable reports concerning it give it first place in the consideration of a test for general use.

6. Variation in antigen is the greatest single cause for disagreement in laboratory results and we believe that a forward step would be the establishment of a standard antigen which might be easier of accomplishment than the uniform adoption of a complete test.

7. Some syphilitic serums give a positive Wassermann reaction with cholesterinized antigen and a negative with acetone-insoluble antigen, and some give a positive result with acetone-insoluble and a negative with cholesterinized antigen.

(*For discussion see p.* 493.)

THE APPARENT SPECIFIC ACTION OF CHOLESTERIN REINFORCED ANTIGEN IN THE WASSERMANN REACTION*

By Edwin Sartain Gault, A.M., M.D., Philadelphia, Pa.

THE universal adoption of the Wassermann as a test for the presence of syphilis has caused considerable research among serologists with the view to standardize the reaction and increase its delicacy and specificity. The importance of the selection of the proper antigen in this procedure has long been recognized and has been the subject of much controversy. This is due to the fact that the best results have been obtained from antigen made from various lipoidal substances which are not specific in their action, but which have the unusual ability of fixing large amounts of complement in the presence of the luetic serum. It has, therefore, been a matter of experimentation as to what lipoid or combination of lipoids is best suited as an antigen or antigens in the reaction. It is well-known also that the Wassermann with the present known antigens is not specific in its action, but gives positive results in frambesia and leprosy. The rarity of these conditions, however, with their marked clinical symptoms make the test practically specific for syphilis.

Since Sach's[1] original experimental reinforcement of alcoholic extracts of beef heart with cholesterin this antigen has received considerable attention. Its sensitive and reliable qualities have placed it in the forefront and it is considered by many to be the best known antigen. There are those, however, who contend that it is too delicate, frequently giving false positives or pseudoreactions.

With a view to obtaining a single antigen which would combine the qualities of accuracy, delicacy and specificity I have used several antigens and a cholesterinized extract in a series of 2207 cases.

My purpose in this paper will be:

1. To study the relative sensitiveness of the cholesterin antigen as compared with other well-known extracts recognized by all as reliable standard antigens.

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2. To review briefly the clinical aspects of all cases in which the cholesterin antigen reacted positively with others negative.

3. To endorse a quantitative method of estimating the fixation power of luetic serum as based on such a standard cholesterin antigen.

CLINICAL MATERIAL

This paper derives its data from a series of Wassermann reactions performed upon cases in the wards of the Sacred Heart Hospital* and includes a wide variety of conditions; the Wassermanns being a routine examination. In addition several hundred private and a smaller number of dispensary cases were tested. Of the 2207 serums tested, 248 were positive reactions varying in their intensity as follows:

41 positive +
13 positive ++
22 positive +++
180 positive ++++

Antigens.—The antigens used in these examinations were three varieties, namely, an alcoholic extract of beef heart, an extract of acetone insoluble lipoids of beef heart and an alcoholic extract of beef heart reinforced with four-tenths per cent of pure cholesterin. These antigens were prepared in accordance with Kolmer's technic.[2]

Technic.—The blood was obtained by venous puncture, bleeding into a sterile tube following which it was then placed in an inspissator, later the clot was broken up, the serum pipetted off and placed on the ice. The Wassermann test was carried out according to the multiple antigen method; the procedure being somewhat as follows:

Serum inactivated from twenty to thirty minutes in a water-bath at 56°. Sheep cells prepared in the usual way and used in a 6¼ per cent suspension. Complement prepared in a dilution of from one to twenty. The Wassermann set-up consisted of four tubes for each serum; the tubes to contain plain antigen, acetone insoluble antigen, cholesterin and control respectively. The complement-fixation set-up consisted of one-tenth c.c. of serum, a double dose of antigen and one-half c.c. of complement.

Tubes were then shaken thoroughly and placed on ice for twelve to fourteen hours at the end of which period the hemolytic system was added, using two-tenths c.c. of sheep cells and a double dose of amboceptor. The tubes were then placed in a water-bath at 37° for one hour at which time the preliminary reading was made and the percentage of hemolyzation noted and recorded on the basis of 100 per cent as complete inhibition of hemolysis. The tubes were now placed on ice for six hours and again read to ascertain if the reactions held.

It will be noticed that I have used a double dose of antigen in all of the set-ups which I feel to be an ample factor to avoid false positives. Our antigens were titrated frequently in order to keep the dosage constant. One and a half or two doses of amboceptor based upon the daily titrations were used as an additional guard. Our reactions were in practically all cases

*Sacred Heart Hospital at Allentown, Pa.

sharply defined, and yet, I feel, quite delicate. The results were checked in a number of cases by various laboratories, especially one.*

Cholesterin antigen as compared in sensitivity to acetone insoluble and plain antigen.—It was found that in 177 positive serums all three antigens reacted with apparently equal degree of inhibition of hemolysis.

In 37 serums the cholesterin antigen showed a greater degree of inhibition of hemolysis than either the plain or acetone insoluble antigen.

In 18 serums the cholesterinized antigen showed the greatest degree of inhibition of hemolysis with a lesser degree in the acetone insoluble and no inhibition or complete hemolysis in the plain extract.

In four serums cholesterinized antigen also showed an increased inhibition of hemolysis with a lesser degree in the plain antigen and complete hemolysis in the acetone insoluble.

In 11 cases the cholesterin antigen alone showed a positive reaction with complete hemolysis in the other antigens. In this class also were several others which I was forced to drop owing to lack of clinical data. The remainder will be taken up in detail later in this article.

In all cases the cholesterin antigen showed a greater degree of inhibition of hemolysis than the other two antigens with the exception of three cases in which the acetone insoluble lipoids gave a greater reaction. Two of these were spinal Wassermanns.

It is notable in the above that in all cases but three the cholesterinized antigen showed a greater degree of sensitivity, while the acetone and plain extract varied with different serums.

In no case was the cholesterin antigen negative with the other antigens positive, which shows its absolute reliability.

In reference to the cholesterin extract.—In the 11 cases aforementioned in which the cholesterin reinforced extract gave a positive reaction, the other two antigens being negative, it was found that five or forty-five per cent showed clinical evidence of syphilis, some of the cases having previously been treated. Four others exhibited symptoms strongly suggestive of lues. Therefore, 82 per cent of these cases showed some clinical evidence of syphilis. The remainder were clinically indefinite as regards specific history, due in a large measure to the absence of clinical data and examination.

These cases are cited individually together with their Wassermann reactions.

CASE 128.—Mrs. M. H. Diagnosis: chronic bilateral salpingitis. Operated and pus tubes found. History of syphilis.

Cholesterinized antigen	Acetone insoluble	Plain extract
50%	0%	0%

CASE 60.—Mr. W. K. Diagnosis: cerebral syphilis. Mental symptoms and general arteriosclerosis.

Cholesterinized antigen	Acetone insoluble	Plain extract
100%	0%	0%

*Homeopathic State Hospital, Allentown, Pa.

CASE 606.—Mr. J. L. Diagnosis: Strangulated inguinal hernia. Died of intestinal perforation and general peritonitis. Postmortem showed aortic disease with marked atheroma of the vessels.

Cholesterinized antigen	Acetone insoluble	Plain extract
50%	0%	0%

CASE 528.—Mrs. A. Diagnosis: Aortic disease, syphilis of the kidney. Marked symptoms of heart failure with physical signs in the aortic area.

Cholesterinized antigen	Acetone insoluble	Plain extract
50%	0%	0%

CASE 1656.—Mrs. A. V. Diagnosis: Cystitis. History of luetic infection and treatment of same previous to examination.

Cholesterinized antigen	Acetone insoluble	Plain extract
25%	0%	0%

CASE 52.—Mrs. B. Diagnosis: Pregnancy. One previous miscarriage. No history of venereal disease obtainable.

Cholesterinized antigen	Acetone insoluble	Plain extract
90%	0%	0%

CASE 123.—Mrs. H. W. Diagnosis: Acute suppurative inflammation of the thumb. Poor wound healing, murmur in the aortic area. History incomplete as to venereal infection.

Cholesterinized antigen	Acetone insoluble	Plain extract
90%	0%	0%

CASE 156.—Mr. E. H. Attempted suicide. History of mental disease. No history of venereal infection obtained.

Cholesterinized antigen	Acetone insoluble	Plain extract
25%	0%	0%

CASE 50.—Mr. H. F. Diagnosis: Gastroptosis. Negative venereal history, but the symptoms simulated gastric crisis.

Note: This case passed from our observation before thorough examination could be obtained.

Cholesterinized antigen	Acetone insoluble	Plain extract
100%	0%	0%

CASE 148.—Mrs. R. Diagnosis: Panophthalmia. No history of lues.

Cholesterinized antigen	Acetone insoluble	Plain extract
25%	0%	0%

CASE 322.—Mr. A. M. Diagnosis: Dislocation of the femur. History deficient as to venereal infection.

Cholesterinized antigen	Acetone insoluble	Plain extract
75%	0%	0%

CONCLUSIONS

1. The cholesterinized extract of beef heart antigen exhibited a greater degree of sensitivity than either the plain alcoholic extract of beef heart or the antigen prepared from acetone insoluble lipoids, demonstrated by the fact that in the 177 positive serums the cholesterinized antigen showed an equal or increased degree of inhibition of hemolysis except in three cases.

2. In no case was there a positive reaction obtained by other antigens, with a negative reaction in the cholesterin extract. In other words, in 2207 serums tested the cholesterinized antigen agreed in reaction with the other antigens in all cases except 11. In these cases the cholesterinized antigen alone was positive.

3. In 11 cases the cholesterinized antigen showed its increased specificity or complement-fixing power by a positive reaction where the plain extract and acetone insoluble antigens were negative.

Finally to sum up, I would conclude that the antigen prepared from an alcoholic extract of beef heart reinforced with four-tenths per cent cholesterin produced an extremely sensitive, reliable and highly specific antigen with exceptional powers of fixing large amounts of complement in the presence of a syphilitic antibody; an antigen of unusual constant sensitivity, with little or no danger of false positives when used in the doses herein prescribed; an antigen whose quality makes it preeminent as one to be used as a single antigen in quantitative methods for estimating the complement-fixation power of syphilitic serums.

REFERENCES

[1]Berl. Klin. Wchnschr., 1911, xlviii, 2066.
[2]Kolmer, J. A.: Infection, Immunity and Specific Therapy. Second Edition, pp. 443-448.

A METHOD OF APPROACHING THE CEREBELLUM*

By Gustav Eckstein, Cincinnati, Ohio

HAVING attempted during the past few months upon small mammals to repeat the classic cerebellar ablations, I was troubled, as others have been, by hemorrhage. When one makes the entry high it is the torcular herophili that interrupts; when one makes it to the side it is the lateral sinus. Unless there is much disregard of muscular attachments, moreover, one finds oneself groping in a pit where even a small trephine seems large and heavy. Out of these difficulties there is a simple way.

Whoever approaches the cerebellum below the occipital protuberance is following essentially the technic that Luciani described a generation ago. Luciani divided the soft tissues in the midline, loosed the muscles to either

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side of the protuberance, retracted them, scraped away the periosteum, and by a trephine penetration enlarged with an osteotome completed the exposure. With the heavier skull of the monkey this was easy; it works well in the dog; but when the animal is as small as even a large rabbit the method proves cumbrous in the extreme.

Why in such an animal should one not bite one's way directly from the edges of the foramen magnum? For in the foramen magnum one has a penetration ready to the purpose. There is no need of trephine. The position is

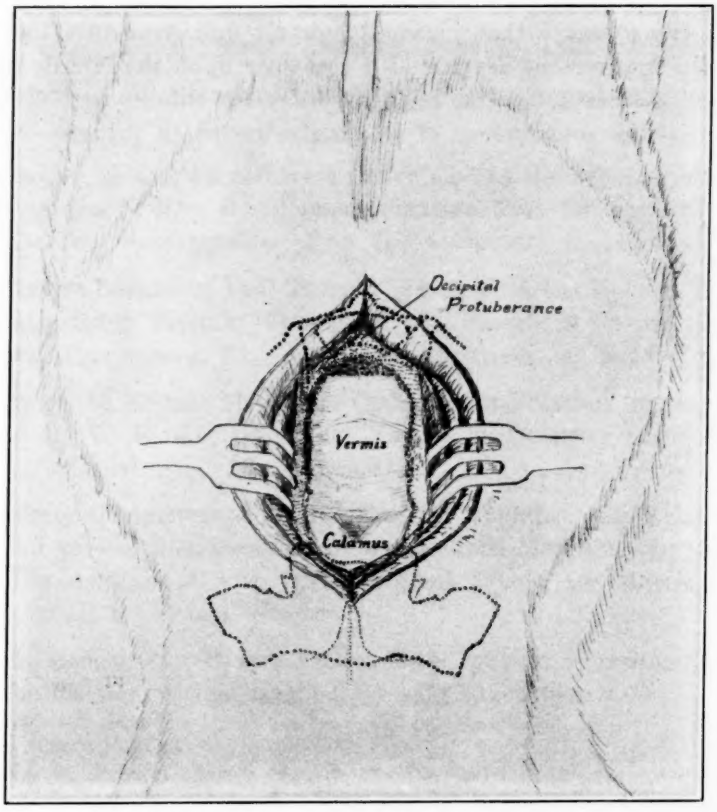

Fig. 1.—The operative field in the rabbit.

accessible, and it secures at once the calamus scriptorius and the lower edge of the cerebellum.

The method is plain. An incision goes down the midline to the bone, and extends caudally to the second cervical vertebra. No muscle insertions are stripped from the superior curved line, the access being ample; and this, when the ablation is for some study of posture, as is likely, becomes an advantage of obvious point. With the occipito-atlantal junction fully in view, the membranous tissue is incised. The anesthetic is withdrawn. The head is sharply flexed. Even in so slight an animal as the nine-weeks' rat the separation resulting from the flexion is sufficient to admit a small biting

forceps. This instrument, gently advanced between dura and bone, continues the exposure. The head need not long be flexed; the anesthetic may quickly be returned; and how far cephalad and laterally the biting shall be pursued depends upon the animal. In the guinea pig one passes safely beyond the forward bend of the vault, in the rat nearly so far, but in the rabbit a too free cut into the heavy bone of the protuberance starts troublesome bleeding. In the sheep, though the occiput above the foramen is thick and channeled, there was in my experience no need even of bone-wax.

Making the approach in this way excites little hemorrhage. There is no severing of insertions for sake of space. But what appears most useful, one avoids the pressure that in small animals quickly grows too great. I have seen the inadvertent bearing of a retractor upon the middle lobe of the cerebellum cause death, and the high mortality for experiments in this region may partly be the consequence of seemingly irrelevant injury.

TRANSACTIONS

PROCEEDINGS OF THE SECOND ANNUAL MEETING OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, SAN FRANCISCO, CALIF., JUNE 25, 26, 1923

(Continued from page 427.)

Third Session, Tuesday, June 26, 1923

Symposium on the Standardization of the Complement-Fixation Reaction for Syphilis.

The symposium was opened by a paper on **The Specificity and Sensitiveness of the Author's New Complement-Fixation Test for Syphilis.** By DR. JOHN A. KOLMER, PHILADELPHIA, PA. (For original article, see page 468.)

Comparative Results of 1400 Tests in Which Both the Kolmer Method and Routine Wassermann Technic Were Used. By MORTIMER COHEN AND SAMUEL R. HAYTHORN, PITTSBURGH, PA. (For original article, see page 476.)

A Study of the Kolmer Method of Complement-Fixation for the Diagnosis of Syphilis. By W. H. KELLOGG, AMY WELLS AND DOROTHY BECK, BERKELEY, CALIF. (For original article, see page 481.)

The Kolmer Complement-Fixation Test for Syphilis: Its Relation to the Clinician and to the Requirements of a Standard Technic. By ROBERT A. KILDUFFE, LOS ANGELES, CALIF. (For original article, see March issue, *The American Journal of Medical Sciences.*)

DR. C. E. RODERICK OF BATTLE CREEK, MICH., gave the results of his experience with the Kolmer method which were very favorable.

DISCUSSION

Dr. Lester J. Palmer, Seattle, Wash.—The Kolmer quantitative complement-fixation test for syphilis was adopted as routine by the Virginia Mason Hospital laboratory August 1, 1922.

The decision to adopt this technic as routine was made following a comparative series of five hundred cases using the new and the old technic. The old technic was a cholesterinized antigen, water-bath method. This comparative series was reported by Palmer and Gibb in December, 1922.

From January, 1922, to June, 1923, 2502 serums were examined. Without giving actual figures the Kolmer technic has proved more sensitive and more specific than our previous method. It has given fewer anticomplementary reactions. The reactions are all clear-cut and easy to read.

In eight cases the reaction varied as to positive or negative on different dates with different serum from the same patient. In four cases we considered the reaction a false positive and in four cases we considered the reaction a false negative.

On two occasions we had trouble because fresh complement had deteriorated, and have now adopted complement, preserved according to Kolmer, as a routine measure.

We have entirely done away with the questionable one and two plus reactions which were so common with our old method, and which so often left us undecided because of the fear of false reactions. In this same class of cases we now obtain clear-cut positive or negative reactions.

In cases under treatment we have found that patients who apparently became Wassermann negative, still gave a positive reaction with the Kolmer technic. For this reason, our cases receive more thorough treatment and ultimate cures will certainly be more frequent than in the past.

It might be emphasized that no deviation from the exact technic as employed by Kolmer will give the same good results—shorter ice-box incubation, water-bath incubation, and other deviations from the exact technic, have proved much less efficient when tried by us.

In summary, we believe that the Kolmer complement-fixation technic is the most efficient Wassermann technic that it has been our experience to employ. We believe that it answers the requirements for an efficient standardized technic, and that much present confusion regarding this reaction in syphilis would be dissipated by its adoption.

Dr. A. H. Sanford, Rochester, Minnesota.—The complement-fixation test that was used at the Mayo Clinic for twelve years, up to January 1, 1923, had the following salient features:

1. Human erythrocyte hemolytic system. Dogs instead of rabbits used for amboceptor to avoid agglutinins. Cells sensitized overnight in ice box and washed once before use the next morning.

2. Guinea pig complement titrated against sensitized cells; double unit used in test.

3. Noguchi antigen used in Noguchi quantities.

4. Patients bled before breakfast on the morning of test. Active serum used. Fixation one hour in incubator. Sensitized cells added. Hemolysis at incubator temperature complete in controls in from fifteen to sixty minutes.

5. Reports sent to physician the same afternoon before 3 P. M.

Beginning July 1, 1922, a comparison was made of the results obtained by our old system and by the Kolmer modification, on blood selected from sixty patients each day. The sixty samples of blood included samples from patients in Dr. Stokes' section, and from patients who had history of syphilis or suggestive symptoms, and who were referred for Wassermann tests, by the general medicine sections. The results of this comparison are shown in Table 1.

TABLE I

Total number of Wassermann tests from July 1, 1922, to December 1, 1922.	15,648	
Comparison of Kolmer test with system employing human erythrocyte hemolysin		
Total number of tests, Kolmer system compared with former routine system	4,740	
Negative agreement (number of tests)	4,070	(86%)
Positive agreement (number of tests)	420	(8.8%)
Disagreement: Kolmer positive, routine negative, 182 (3.8%); routine positive, Kolmer negative, 64 (1.4%)	246	(5.2%)

In certain instances, there was disagreement between the results obtained by the two methods of complement fixation. Dr. Paul O'Leary, of Dr. Stokes' section, considered carefully the history and findings in each case in which at any time the two methods did not give the same result. The analysis of these cases is shown in Table II. It must be noted that in Table I the figures are for tests, whereas in Table II they are for cases.

TABLE II

ANALYSIS OF CASES IN WHICH THERE WAS DISAGREEMENT AT SOME TIME IN THE TWO TESTS

	ROUTINE + KOLMER − CASES	KOLMER + ROUTINE − CASES
Group 1. In which clinical findings warranted a diagnosis of syphilis irrespective of serology	12	67*
Group 2. Where the diagnosis was confirmed after positive Wassermann reaction	2	14
Group 3. Known syphilis under treatment	12	53
Group 4. In which the Wassermann varied from day to day (that is, in Wassermann series)		
Group 5. Positive serology only		
(a) Suspicious history and findings	7	16
(b) False positives (?)	9	4

*Some of these cases had several tests in which the routine test and Kolmer test agreed.

As a result of the comparative study, we were convinced that the new Kolmer method was a more sensitive test than our old method. After a few weeks in which we ran every serum with both methods, but used a qualitative test instead of the full six-tube quantitative test, we adopted a three-tube technic at Kolmer's suggestion. Since January 1, 1923, we have used this last method exclusively, using 0.1 c.c., and 0.05 c.c. quantities in the tube with antigen and 0.05 c.c. in the control tube. We have made 16,772 tests in five months. At present we are not prepared to give any statistics comparing the results from the exclusive use of the three-tube Kolmer method for five months with a similar period when we used our old method. I know, however, that we are getting a higher percentage of positive reactions.

The disadvantages that we have found in the test apply chiefly to our local condition. With the former system we were able to return the report to the physician on the afternoon on which the patient was bled. The report now is delayed twenty-four hours. However, this does not work any very great hardship on the patient in the majority of instances.

Our chief technical difficulties have been in the matter of preparation of salt solution. For some time this has been a great puzzle to us, for although we always use Pyrex glassware, fresh triple distilled water and the purest of sodium chloride in 0.85 per cent solution, we have often found that such salt solutions could not be used, whereas one made with tap water was suitable. Dr. Mason has been working on this problem recently and, while his studies are not complete, we feel that we might call attention to the fact that with a delicate system like the Kolmer modification, the introduction of a buffer in the salt solution is of importance. This, I believe, accounts for the fact that some undistilled water, containing as it often does, the proper amount of natural buffer, will make a suitable salt solution for serologic work, while an unbuffered solution will give unsatisfactory titrations because of very rapid changes in pH value. It is impossible to determine the pH of triple distilled water, using phenyl red as an indicator, as the rapid absorption of carbon dioxide from the air changes the pH completely to the acid side. Consider what must happen when expired air is blown with a pipette through a solution made with such water. I believe that there should be very little deviation from a constant pH_2 which should be on the alkaline side of neutrality, in solutions that are used for serologic work. I would suggest further that, with a system such as we formerly employed, the lack of buffer in the salt solution was not apparent because of the fact that we were using proportionately much more serum in amboceptor, complement and patient's serum, thus adding, without taking it into account, buffers that carry through the reaction. Our suggestion would be to prepare salt solutions containing artificial buffers, especially whenever the serum reagents were used in very small quantities.

Dr. W. P. Butler, Shreveport, Louisiana.—I have no paper prepared, but I want to make a few remarks on this subject. We have not run enough tests in our laboratory to make a report of very great value. We have had only about 700 cases since we started

to use this technic, and of this number 476 have been run parallel to as many as three other tests. Our aim has been to run three antigens or three different technics, and of this number (476) we have run the Kolmer test parallel with these other three. The cholestrin antigen and acetone insoluble antigen are not so sensitive and consequently do not give so many positives. The Kolmer test is more sensitive but does not give false positives, as shown by the case histories. The Kolmer method gives a positive reaction earlier in cases than any other method and is therefore very valuable. The earliest positive we have had is nine days. Many treated cases show positive by Kolmer method when other methods give negatives. We do not care for the quantitative test on account of the time involved in making the reading scales, and the fact that to an experienced worker a four-plus will always be a four-plus and a two-plus will always be a two-plus. After hearing Kolmer's explanation of the value of the quantitative test I am convinced that it should be used. Of the three methods used along with the Kolmer method, 416 correspond to the Kolmer method, and only 60 do not correspond, bringing this down to a percentage basis that makes 87.4 per cent which did correspond. We have only had a few spinal fluids, and we found more positives, something like 50 per cent, by using the Kolmer method.

Dr. N. D. Goodhue, Dayton, Ohio.—In our study of 1000 cases, comparing the Kolmer method with a modified Human-Craig system, we noted a disagreement in the strength of the reaction of 3.3%.

The Craig system used differed from the Kolmer method in but two respects; namely, that the antigens were different, and that a human hemolytic system was employed. In this system, we used two units of amboceptor, two units of complement, and the titrations for the same were carried out in much the same way as in the Kolmer technic. The total volume of the test was the same—a 2% suspension of cells was employed, and the pipetting was essentially the same. Two antigens were employed; a sensitive cholesterinized antigen and a plain alcoholic ox heart antigen. The antigens were made up according to the method described by Hinton of Boston. Briefly, this is as follows:

Small pieces of lean heart muscle are placed in mason jar for two or three weeks covered with 95 per cent alcohol. Muscle is then ground, and a 1/10 alcoholic suspension of this is put into an incubator at 37° C. for two or three weeks, and shaken daily. Then 100 c.c. of this alcoholic extract is saturated with cholesterin. When saturated, 100 c.c. of plain alcohol is added, and this makes the stock solution. This is titrated in much the same manner as the Kolmer antigen is titrated, that is, 10 units are used in the test if the antigen does not show any hemolytic or anticomplementary difficulties.

Dr. E. H. Ruediger, Bismarck, North Dakota.—It seems to me that the first thing to be done in trying to standardize the Wassermann test is to preserve a standard. If we have a suitable preserved standard, we can test the same serum any number of times, and the same serum can be tested by any number of persons any number of times, because the same serum can be sent a long distance without any material injury to the serum. It is especially valuable in teaching institutions where they can always have a number of positive serums. You do not have to go to the clinician for serum every time you want serum for teaching purposes. I have been working on the preservation of serum for the past seven years, and I have found it very satisfactory. The suitable methods are three. The most suitable is perhaps drying the serum, putting it in glass tubes under vacuum. Such a serum keeps perfectly according to my experience. Another method is to mix the serum with glycerine, and keep it at a temperature of approximately 0° F. Now, there is a drawback to this method, because many of you would not be able to get this temperature. In most of the places, we can get such a temperature. I was in the same position once, I was anxious to try out this method, but I was not able to get the temperature. Finally, after two years of effort, I made the acquaintance of ice cream manufacturers. They have a temperature ranging about thirty below zero. I have been keeping it now in the ice cream room about three years. The temperature is not very constant, but it never goes above zero Fahrenheit, and the lowest I have seen it was thirty below, and at this tem-

perature I am not able to detect any deterioration in the course of three years. This is not true of complement. It usually deteriorated in two months. The best I have done so far was ten weeks. But in human serum, I cannot detect any deterioration in three years. Another method is just to take a mixture of glycerine and serum and keep it at a temperature of nearly freezing, say 1° C. This temperature can be had at any place. All complement serums that fall below a certain standard are rejected, and only such serum used as comes up to a certain standard. Primary incubation is at approximately 1° C., because that is a temperature that will preserve the complement. The period of incubation is five hours. Before the sensitized cells are added, they are placed in a warm water-bath about ten minutes so that hemolysis will take place, because hemolysis does not take place in a cold water-bath; this method I have been using for the last five years without any trouble at all. Every human serum is subjected to a quantitative titration. The quantitative titration I have used is very simple. The human serum is diluted, and the result obtained is multiplied by the dilution. The quantitative titration, of course, takes up some time, and many laboratory workers will object because of the extra work. I started in doing the quantitative test some years ago. If I now submit a qualitative report, the clinicians return the report and ask, "How strongly positive?" They absolutely will not accept any but quantitative reports.

Dr. Z. E. Bolin, San Francisco.—I have listened to Dr. Kolmer and the rest of the men, and I have read very carefully all of Dr. Kolmer's paper. The thing that strikes me about it is that from here on the serologists' bible will be that list of thirty-two papers, because it has covered most completely every factor that I know of in the Wassermann test. One point which I discovered, not only in the Wassermann, but in other tests besides Kolmer's, is a factor which I call the protein factor, and can easily be demonstrated by anyone who wishes to do so. It has been handled. Dr. Kolmer has handled it, and several other people have this morning shown how a smaller dilution or a smaller amount of serum gives a stronger positive than a larger amount. It can easily be demonstrated in the titration of the complement. In the titration of hemolysin with the addition of human serum, it gives a lower unit than the same titration with the same reagents without the addition of human serum. Now, this factor should be taken into consideration, because in my experience it has occurred in most of the human serum that I have tried. In some 60 to 70 per cent of the human serum, it will give this added hemolytic factor. I hope the serologists may work on this, and get that protein factor. Dr. Sanford mentioned the buffer effect on the reaction. There might be something in the buffering of it.

Dr. Walter V. Brem, Los Angeles.—When we begin to train new technicians in our Wassermann test, even if they have had experience elsewhere, we find that it takes a number of weeks to adjust them to our technic. Dr. Kolmer's technic is considerably more complicated than ours, and, therefore, it would take, I think, considerably longer for one to become proficient in it. It would not be fair, therefore, to draw conclusions from parallel series of cases until one has worked with this technic for quite a long while. I hesitate, therefore, to discuss this question, because I think we have not gone far enough in mastering the Kolmer technic. Eventually the question must be decided upon parallel tests controlled carefully by clinical findings.

When Walker and Swift first wrote on the cholesterinized antigen, recommending the 0.4 per cent emulsion, we began to use it at once, but found we got nonspecific reactions with it, and that it was not a safe antigen to use. We then found the strongest nonspecific fixing sera possible, and with them, we tested different concentrations of cholesterin in the antigen. We found that a 0.1 per cent cholesterinized antigen was a perfectly safe antigen, giving no nonspecific fixations, and that at the same time it was quite sensitive—sensitive enough, we believed to use in the test. Therefore, we have used the 0.1 per cent cholesterinized human heart antigen since 1915, and we have made over one hundred thousand tests with it.

I learned how to do the Wassermann test originally from Dr. Albert Keidel, to whom

I wish to acknowledge a debt of gratitude. I have not changed it very much since then except to introduce the 0.1 per cent cholesterinized antigen, and the ice box fixation.

Now, the parallel tests we have run so far with Dr. Kolmer's test and our own, indicate that ours is a more sensitive test than his. We have had in a series of 127 parallel tests, eight in which our test gave a four-plus positive reaction, and the Kolmer test was negative (2) or less than four-plus (6). In eleven instances, our test gave a one-plus reaction while the Kolmer test was negative. In two instances, Kolmer's test was two-plus while our test was one-plus. In one instance, ours was three-plus and Kolmer's one-plus. There were, then, 22 discrepancies, in 20 of which our test was stronger. Of course, the question arises "Did the Kolmer-negative patients have syphilis?" and the criticism may be made that our test gave nonspecific fixations. But after our long experience with it, we do not believe that such is the case. Since our test is also much simpler, we would be very loath at the present time to exchange it for Dr. Kolmer's test.

I realize, of course, the great advantage of a standard test for all our laboratories, because when we have not a standard test, the discrepancies tend to discredit the test.

With reference to the quantitative test, we have been titrating our strongly fixing sera for a good many years, and we feel that it is of great value. Our quantitative test is not exactly like Dr. Kolmer's, but it is designed to give the same information, and it is of the utmost importance.

Dr. F. W. Hartman, Detroit, Michigan.—One point which Dr. Kolmer brought up was the matter of electric incubators in connection with his tests, and it seems to me that this is very important, especially if you do not have other facilities. It seems to me that that suggestion has been overlooked. I think in comparing these tests, we should include temperature at the primary incubation, and whether that was a temperature that was likely to vary.

Dr. Albert Keidel, Baltimore, Md.—I just want to say one or two words in reference to the remarks about the length of time it takes to perform the Wassermann and the Kolmer technic, and also, those remarks that have been made with reference to the temperature at which these tests have been made. I want to ask Dr. Kolmer whether he has considered at all a method of cold incubation described in which the primary incubations were carried on in an ice bath? By means of the ice bath, we were able to demonstrate that the amount of fixation that would occur, takes place in one hour, and that when the tests were carried along in that bath for longer than one hour, they got no more fixation. In other words, it is possible to reach the limit of fixation at 0° C. in an ice bath in one hour. It seems to me that unless there are some objections to it about which Kolmer knows, he might possibly be in a position to revise his technic.

Dr. Truman C. Terrell, Fort Worth, Texas.—We have been using Kolmer's system since last September. We have also modified the human system to practically the same technic as the Kolmer system. We run both systems, using 3 antigens each, Kolmer's antigen as one of them, and the 0.2 per cent cholesterinized antigen and the plain alcoholic antigen as the others. We find we do pick up bloods that are anticomplementary with the sheep system that are not with the human system, thus saving time. The antigens we have been using since 1918, are made as follows:

Take three or more big beef hearts, remove as nearly as possible all fats and grind in meat grinder, spread out on a large glass and dry with fan, and then grind with a cottonseed meal grinder until we have a very fine powder. Then take a wide mouth pound bottle, and put in 3 inches of this powder, and cover with ether, and extract the fats. Draw off the ether, and replace until the ether is colorless, then add sufficient amount of alcohol to cover the powdered beef heart approximately 1 to 1½ inches, shake thoroughly, and extract in incubator for a number of days. This alcoholic extract is used in making 0.2 per cent cholesterinized antigen as well as the acetone insoluble extract. We have found that the antigens made in this manner are very low in anticomplementary power, hemolytic power, and high in antigenic power. The acetone insoluble antigen is very much

like Dr. Kolmer's antigen, when cholesterinized, the difference being that the ethereal extracts were first discarded.

In the last series of Wassermanns, we ran 914. Of this series, 899 checked with all antigens both systems. Of the 15 not checked, 3 were anticomplementary by the sheep system, and 4-plus positive by the human system, and one 4-plus positive by the sheep system and negative by the human system. Other than this, the variations were slight in the remaining number that did not check. In another series of 110 Wassermanns using some antigen sent by Kolmer, and antigen made according to Kolmer; as well as using the alcoholic extract and the cholesterinized antigen, all checked except one, which was anticomplementary with sheep system, and 4-plus positive with the human system.

Dr. Robert A. Kilduffe, Pittsburgh, Pa.—I would like to say a few words in regard to the use of the ice water bath. I used that technic after checking up with a series of about four or five hundred patients, and as far as the qualitative test goes, the question of 4-plus of positives or negatives one hour in the ice water bath, the temperature of which can be regulated by the addition of brine, the results checked up perfectly. In using Kolmer's quantitative method, it seems to me in a relatively small series the results were better in the higher dilutions of the serum if the incubation period was increased to not less than four hours. The results of varying periods of primary incubation depend entirely upon the amount of reagin in the serum tested.

Dr. William G. Exton, New York.—It has been customary with some people who have had syphilis, in applying for life insurance, to either forget they have had it, or to call it by some other name. It has been usual for life insurance companies to ask them to have a Wassermann test, preferably at some local laboratory. I have had three hundred such cases, and I have not had one positive reaction report from any laboratory, and these laboratories are scattered throughout the country. I have written to a number of these laboratories asking them what technic they used, and invariably the answer is this: "We use our own modification of the Kolmer or our own modification of Noguchi," or some one else. No one seems to run a straight technic. Now, in contrast to this, we started a conservation service for our policy holders, and not being able to do a Wassermann at the home office laboratory, we had it done at a number of outside laboratories, and I might say we got about 90 per cent positive reports. I don't want to go into the psychology of the matter at all, but I do think that you men who are running laboratories throughout the United States have got to get together, and decide what is the one. In our short experience, we have staked our reputation and money on the Kolmer test, and we think that some one test, whether it is the perfect one or not, has got to be made standard, unless the laboratories are going to be disregarded more or less.

Dr. John A. Kolmer, (closing).—It is scarcely necessary for me to state that I am highly gratified with the favorable reports rendered this morning on my new antigen and new complement-fixation test for syphilis.

Naturally I am mostly interested in learning of possible difficulties and defects, as our experience with the method has been so uniformly satisfactory.

I trust that my attitude towards standardization is properly understood. I believe standardization of technic is advisable for several reasons provided the technic proposed for a standardized test possesses the maximum degree of sensitiveness and freedom from nonspecific reactions. I have striven to attain these qualities in our method and trust that the experiences of other serologists with the method will be as satisfactory as our own. I have a great faith in the reliability and value of the complement-fixation test for syphilis but believe it is necessary for the serologists of this country to check up their method or adopt some other technic to secure as highly sensitive and specific reactions as possible as their contribution toward aiding in the diagnosis and eradication of a disease possessing tremendous economic importance.

Time will not permit me to discuss in detail all of the papers and reports, but a few points are worthy of special attention.

Doctor Kellogg has very properly referred to variation in results due to different antigens. In my opinion differences in the kind and dosage of antigen constitute the most important single factor of variation in the syphilis complement-fixation test. I believe that the lipodophilic reagin in the sera of different syphilitic individuals may vary in their complement-fixing power with the lipoids of different extracts. For this reason I have advocated that the antigen be prepared by extracting the muscle of six or more beef hearts instead of one. The Digestive Ferments Company have prepared to furnish the desiccated and pulverized muscle made up from a large number of hearts and in my opinion, a polytropic extract of this kind will fulfill this requirement.

The subject of nonspecific reactions due to cholesterolized antigens was very properly mentioned. I believe that with extracts saturated with cholesterol (approximately 0.4 per cent) such reactions may sometimes occur, but I could not escape the conclusion that some cholesterol is required to render the antigen sufficiently sensitive. For this purpose, however, 0.2 per cent cholesterol is sufficient and is the amount used in preparing our antigen. To the best of my knowledge this antigen has not yielded any nonspecific reactions—certainly not in our own experience.

The interesting paper by Keidel and Kemp furnishes data in my opinion supporting the hypothesis that the administration of antiluetic drugs may provoke a positive complement-fixation reaction by stimulating spirochetes into activity. I am convinced, however, that no drug including arsphenamine and neoarsphenamine, administered to a nonsyphilitic individual ever engenders the production of the substance yielding positive Wassermann reactions. The fluctuations in degree of complement-fixation reported by them indicates either that the treatment may be provocative, or that the antibody content fluctuates as reported by Craig. Very probably both factors were responsible for the fluctuations in the degree of complement fixation observed by them; similar results have been noted by Greenbaum in the clinic of Schamberg in Philadelphia.

I heartily agree with Kilduffe in his remarks concerning the advisability and necessity for reeducating a large number of the medical profession in the value and limitations of the Wassermann reaction. I wish also to heartily endorse the remarks of Dowling and hope that any who give our methods a trial will conduct the tests exactly as described in every detail and resist the temptation to make modifications, however slight they may appear, until at least the method as described has received a fair trial.

Sanford's remarks on the buffer salts in relation to the saline solution are of considerable importance because a few serologists have experienced difficulties with the hemolytic system. It may be too, that these buffers in serum may be responsible for the weaker reactions sometimes observed with the 0.1 c.c. dose as compared with the 0.05 c.c. and smaller amounts. Naturally one first thinks that native antisheep hemolysin may be responsible but I am quite sure that this is not the case in all instances. I suspect that something else in the serum deviates the complement or prevents its fixation by syphilis reagin and the antigen. This may have a relation to the buffers and is deserving of investigation. Whatever may be the explanation, however, the occurrence of the phenomenon emphasizes the advisability and indeed, the necessity, for conducting each test with varying amounts of serum in order to secure the best results.

Relative to the preservation of serum with glycerol mentioned by Ruediger, I beg to state that with our new method the technic has not proved satisfactory owing to the anticomplementary properties of the mixture of serum and glycerol. We have long been interested in the preservation of serum by drying, with glycerol, in a frozen state, etc., and found freezing to yield best results. Our work was conducted in connection with studies aiming to furnish a standard unit for the titration of antigen. For this purpose we have found it better to preserve a standard antigen for titration purposes and have worked out a method whereby antigen can be standardized by a central laboratory or sent out as a standard unit for the titration of antigens in individual laboratories. Supplying antigen from a central laboratory for complement-fixation tests would alone constitute a real step in advance and I trust that arrangements along this line will be made as has already been done in Germany. This society itself could take the initiatory step.

Hartman, Sanford, Keidel and Kilduffe have brought up the question of temperature and duration of primary incubation. In our work a primary incubation of 1 hour in a refrigerator or cold water-bath has not given us as good results as 16 to 18 hours at 6 to 8° C. I wish it were otherwise and that the time specified for the primary incubation in our test could be shortened to 4 hours or less, but our experiments show that *the longer period is required for the most sensitive reactions with the smaller amounts of serum.* With strongly positive sera employed in 0.2 or 0.1 c.c. amounts, complement fixation occurs very rapidly at most any temperature from 0 to 38° C. but this is not true when smaller doses of such serum is employed in a quantitative test such as ours or when weakly positive sera are employed. In Philadelphia we have no difficulty in maintaining a temperature of 6 to 8° C. in the lower compartments of a good refrigerator kept well filled with ice. As mentioned by Sanford, however, the electrical refrigerators now available are particularly satisfactory.

I was very much interested in Terrell's remarks concerning the antihuman hemolytic system. In our new method this system may be employed, but the results in our hands have not been so good as observed in comparative tests with the antisheep system. The difference is due to the lower potency of antihuman hemolysin requiring the use of 1:10 or 1:15 dilutions of complement instead of 1:30 in order to secure a satisfactory hemolytic system. Our experiments have shown that tests conducted with 1:10 complement are not so sensitive as tests conducted with 1:30 complement and I believe the differences are due to the presence of serum constituents in the larger dose of complement serum (1:10 dilution) capable of interfering with the fixation of complement in exactly the same manner as 0.1 c.c. of a patient's serum may yield a weaker reaction than 0.05 c.c. I believe that it is a principle of complement fixation that up to a certain minimal level, the smaller the amount of complement used the more sensitive are the reactions and since antisheep hemolysin is readily made in such strength that the complement can be used in 1:30 dilution that this explains the superior sensitiveness of the antisheep system.

Fourth Session—June 26, 1923—2:00 P.M.

The Board of Censors presented a report recommending a list of applicants for membership which was unanimously adopted.

A lengthy discussion ensued on the question of raising the ethical status of the Clinical Pathologist and the advertising of commercial clinical laboratories. Participating in the discussion were Drs. Josiah Moore, Chicago; Ward Burdick, Denver; Wm. C. MacCarty, Rochester; E. A. Victors, San Francisco; Walter P. Brem, Los Angeles; Frank W. Hartman, Detroit; C. E. Roderick, Battle Creek; R. A. Kilduffe, Los Angeles; E. H. Ruediger, Bismarck, N. D.; Dr. Harriet Lawrence, Portland, Ore.; Dr. Clara Israeli, Concord, N. H.; Dr. Frank B. Stephenson, Denver.

The general sentiment was in favor of upholding the constitutional provisions of the Code of Ethics which reads as follows:*

Article VIII—Code of Ethics

Section 1. The Code of Ethics of this society shall be the same as that of the American Medical Association.

Sec. 2. It is deemed unethical to publish advertisements in medical journals calling attention to the merits of a particular laboratory or announcing the fees for laboratory examinations.

Sec. 3. It shall be deemed unethical for a member of the society to lend his name for publication in the laboratory advertisements violating this code of ethics.

*During the session, a committee from the American Society of Clinical Pathologists, consisting of Drs. Ernst Victors, Wm. C. MacCarty and Philip Hillkowitz were summoned to a conference with the Trustees of the American Medical Association in order to present the views of the A. S. C. P. on advertising of commercial laboratories in the Journal.

The nominating committee brought in the following names of officers for the ensuing year to be voted on by the Society:

For President—Dr. William C. MacCarty, Rochester, Minn.

For First Vice President—Dr. John C. Kolmer, Philadelphia, Pa.

For Second Vice President—Dr. George Ives, St. Louis, Mo.

For Secretary-Treasurer—Dr. Ward Burdick, Denver, Colo.

On motion the above officers were unanimously elected.

The incoming president in his speech of acceptance emphasized the responsibility of the clinical pathologist and the high ideals which he should strive to attain.

It was moved and carried to accept the invitation of Dr. MacCarty to hold the next convention in Rochester, Minn., in the week prior to the meeting of the American Medical Association.

The meeting concluded with the adoption of a motion to accept the Kolmer method of complement-fixation reaction for syphilis as an official standard method of the American Society of Clinical Pathologists.

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EDITORIALS

Adjunct Therapy in Neurosyphilis

NEITHER salvarsan nor mercury has proved highly efficient in the destruction of the Treponema pallidum, once it has become firmly entrenched within the central nervous system. According to prevalent conceptions, this tissue is relatively less susceptible to invasion, but having once been involved it shows less ability to provide local defense reactions. An additional factor is the relatively low penetrability of nervous tissues to therapeutic agents. It is true that many neurosyphilitics respond satisfactorily to ordinary routine antileutic therapy, but while they may evidence temporary improvement, the majority retrogress. This is particularly true with regard to general paresis.

Silver-salvarsan has not attained wide acceptance. Its use is based upon the hypothetical mordant properties of silver, serving more firmly to fix the arsenical within the cell substance. Most syphilographers still rely, by preference, upon the coincident or alternate use of mercury with salvarsan or neosalvarsan. More recently bismuth, either as metallic bismuth or in combination as a salt, has been substituted for mercury with apparently comparable results. Bismuth appears to penetrate into the spinal fluid more readily than

mercury. The Swift-Ellis procedure appears to remain, however, the most generally accepted and the most generally efficient plan of treatment in neurosyphilis. Many failures, however, follow its use.

Sulpharsphenamine, a compound prepared from arsphenamine, formaldehyde and sodium bisulphite and differing from neoarsphenamine only in a side chain containing one additional atom of oxygen, is much more stable than neoarsphenamine or arsphenamine both in dry form and in solution. Its arsenic content is 22 per cent as contrasted with 30 per cent for arsphenamine. Voegtlin finds it of decidedly lower toxicity and of more constant spirocheticidal power. The variability in spirocheticidal action of different lots of neoarsphenamine is well known. From studies in trypanosomiasis sulpharsphenamine would appear to penetrate into the spinal fluid more effectively than either arsphenamine or neoarsphenamine, and it has, therefore, been seriously considered for use in the treatment of neurosyphilis.

Halloran has used sulpharsphenamine in twelve cases of advanced general paralysis and one of cerebrospinal lues. Scarcely sufficient time had elapsed when the author reported his series to draw conclusive deductions. All cases showed some slight physical improvement, the majority gaining anywhere from three to nineteen pounds in weight. During the six weeks of treatment there was little change in the mental state. The blood Wassermann of one patient and the spinal fluid of another became negative. Another positive blood and a spinal fluid became less strongly positive. The remainder showed no diminution in either blood or spinal fluid reaction. All of the paretics showed decreases in the spinal fluid cell count, ten being reduced practically to normal. Analagous changes were noted in the spinal fluid albumin and globulin content and in the colloidal gold curve. Although these results are not conclusive it may be that further investigation with sulpharsphenamine will elicit indications of its value in cerebrospinal syphilis.

Flumerin is a mercury compound, the disodium salt of hydroxymercurifluorescein. Like mercury it possesses relatively low spirocheticidal properties. Its pharmacologic action appears to be similar to that of mercury in that, when tolerance has been exceeded, flumerin will produce stomatitis and enteritis. Like mercury, its exact action in the healing of syphilis is not clearly understood. Moore and Wassermann claim one distinct advantage over mercury, namely, a high therapeutic ratio. This drug is recommended for use in alternation with arsphenamine in the treatment of early syphilis. The results in neurosyphilis and in latent syphilis have shown no advantage over other active mercurial compounds.

Tryparsamide, first thoroughly studied in the treatment of trypanosomiasis by Pearce, has received rather extensive investigation within the last year in a selected group of clinics, and reports are now appearing as to its value in neurosyphilis. This drug is by no means as active a spirocheticide as arsphenamine or neoarsphenamine. Even its trypanocidal index is but one-fourth to one-third that of these other drugs. Two properties possessed by tryparsamide have suggested its possible value in therapeutics, namely, its high penetrability, and its general tonic action. As a spirocheticide,

tryparsamide is distinctly inferior to salvarsan. Its use in early syphilis, where the aim must be to destroy the invading host, is therefore clearly not indicated. In latent syphilis and particularly where the nervous system has become involved, there is less probability of complete destruction of the spirochetes and attempts to improve the patient's general condition become of paramount importance. There is some natural tendency toward spontaneous recovery in syphilis. Often, to be sure, such recovery is not complete and the disease becomes latent. A drug such as tryparsamide acting primarily upon the body tissues, improving the nutrition and increasing the natural defenses against the spirochete, is then clearly indicated.

According to present concepts, central nervous system invasion is primarily a matter of defective or ineffectual resistance, and it is this in particular that tryparsamide is supposed to enhance. When in addition to this tonic action the drug has some spirocheticidal activity, and when because of its easy penetration into the tissues such activity is fairly well utilized, it becomes a particularly desirable remedy.

Patients under treatment have shown remarkable clinical improvement. One girl, aged twenty-four, with congenital syphilis, during the first four weeks of tryparsamide treatment gained thirteen pounds, even though her weight had remained constant under antecedent arsphenamine therapy. Lorenz and his collaborators, and Moore with his associates report comparable results. Parenchymatous neurosyphilis was clinically apparently arrested in nearly two-thirds of the series, and improvement was noted in an additional fifteen per cent. In meningovascular neurosyphilis 90 per cent were clinically arrested, with the other 10 per cent improved. The blood Wassermann reaction became negative in 83 per cent of Lorenz' series and in 6.6 per cent of Moore's. It was reduced in 15 per cent of Lorenz' and in 20 per cent of Moore's cases of parenchymatous neurosyphilis. Entirely comparable results were obtained in the spinal fluid findings.

Marie and Kohen have treated general paralysis by intramuscular injections of insoluble salts of bismuth, combined with subcutaneous injections of tuberculin. Bismuth penetrates the cerebrospinal fluid more rapidly than either mercury or arsphenamine and was therefore used by preference. The action of tuberculin appears to be nonspecific, similar to that obtained in the treatment of general paresis with experimental malaria infection. Of twenty cases eleven presented improvement of sufficient degree to be termed remission. The Argyll Robertson pupillary reaction, the tremor, the speech and memory defects disappeared to such an extent that the patients returned to work. Two were slightly improved while five advanced cases remained unimproved. Three tabetics previously treated with bismuth alone, with no improvement other than diminution in the frequency of tabetic pains, experienced complete remission of symptoms under combined bismuth and tuberculin treatment, and in them the spinal fluid Wassermann became negative.

It is of interest to observe in this review that up to the present time no more potent spirocheticide suitable for clinical use has been found than

arsphenamine, and that for increasing therapeutic efficiency drugs are being investigated whose action upon the parasite itself is to a great extent of secondary importance.

REFERENCES

Marie and Kohen: Bulletin de la Societé de Therapeutique, November 14, 1923.
Brown and Pearce: Jour. Am. Med. Assn., 1924, lxxxi, 5.
Moore and Wassermann: Ibid, 1923, lxxxi, 1840.
Halloran: Boston Med. and Surg. Jour., 1924, exc, 55.
Moore, Robinson, Keidel: Jour. Am. Med. Assn., 1924, lxxxii, 528.

—*W. T. V.*

Hemopoietic Action of Germanium Dioxide

CONSIDERABLE attention has recently been devoted to the stimulating effect of germanium dioxide on bone marrow, in the treatment of both primary and secondary anemias. Most results reported are distinctly favorable to this new drug. An article by Schmitz in the March number of this journal gives a brief historical resumé of the experimental work with germanium dioxide. Hammet, Nowrey, Müller, Iszard and Lenker, have described erythrocythemias following the administration of germanium dioxide and report favorable results in the treatment of both primary and secondary anemias. Müller and Iszard find a level of saturation in the blood above which excess of germanium dioxide appears not to be utilized. Blood apparently can absorb only about 0.014 per cent of its weight of the chemical. From chemical and spectroscopic studies they conclude that germanium in the blood is distributed in part in the red blood cells and in part dissolved in the plasma, and that it probably serves as an oxygen carrier similar to hemoglobin. Apparently it exists in arterial plasma as the dioxide being reduced in the tissues to germanous oxide and returning to the lungs chiefly within the erythrocytes.

Kast, Croll and Schmitz conclude from clinical investigation that germanium dioxide exerts a distinct "erythropoietic action" and while having some value in pernicious anemia, is particularly useful in secondary anemia. They appear to be rather less highly enthusiastic than are Hammett and his associates.

Minot and Sampson take exception to the experimental work of these various observers in pointing out that the criteria chosen for demonstrating erythropoiesis are not satisfactorily rigid. A simple increase in the total red cell count after the administration of any drug is not conclusive, particularly when the experimental work is done on laboratory animals in which there is normally considerable fluctuation in the red count. Increased hyperemia of the bone marrow is likewise not a reliable criterion. Even increase in the number of nucleated red cells in the marrow is not conclusive unless the difference between the control marrow and that of the test animal be very great.

Minot and Sampson have followed the total red counts in a series of untreated rabbits, and in rabbits receiving germanium dioxide, and while

the fluctuation has been great, it was no more pronounced in the treated animals than in the controls. In fact among the six control rabbits they observed a difference between the highest and lowest red cell counts of 3,000,000 per cubic millimeter. Marked alteration in the level of the red cells often developed rapidly and occasionally extreme variation occurred within as short a period as three days. They point out a similarity to the well-known fluctuations in temperature of apparently normal rabbits. The hemoglobin fluctuated with the red cell count, but to a lesser degree. Occasionally they observed increases in total red count following the administration of germanium, but these were quite evanescent and entirely comparable to similar fluctuations in control animals.

The observers noted no increase following treatment in reticulated red cells in the peripheral circulation. Fluctuations in the total white count were similar to those of the erythrocytes. Two distinct increases above normal following treatment persisted only for a few days. No significant or characteristic change was observed in the differential count except that with the highest counts, the polynuclears were slightly increased and there was a doubtful tendency for these forms to increase during one or two days after the second or third injections of germanium. At no time was any increase of immature white cells observed. There was no notable change in the platelets during or following treatment.

Eight patients were followed, two cases of pernicious anemia, one of severe purpura hemorrhagica, one of chronic lymphatic leukemia, two of anemia due to chronic blood loss, one of cancer of the stomach and one of chronic focal sepsis. In none of these eight was any significant alteration observed in the numbers of reticulated red cells, platelets or bone marrow white cells, or in the character of the bone marrow elements, suggestive of increased marrow activity following the taking of germanium.

No increase in red count or hemoglobin percentage was noted in the cases of pernicious anemia, in purpura hemorrhagica or in lymphatic leukemia, but transient increases were noted in the remaining cases. However, these increases appeared no more pronounced than has often been the case in individuals not treated with germanium dioxide.

Minot and Sampson conclude that germanium dioxide may induce a transient increase of the red cell count of the peripheral blood, but that no evidence has been adduced that it is a powerful erythropoietic stimulant. The four cases with transient abrupt red cell elevations improved no faster nor were they influenced more beneficially, than other cases of the same type not treated with germanium.

Alexander has observed no clinical improvement nor increase of hemoglobin or erythrocytes in three cases of pernicious anemia following the administration of germanium by mouth, or hypodermically over periods of three to four weeks. All cases received the drug in the dosage recommended by earlier investigators.

A clinical point that might well have been emphasized by the critics of

germanium dioxide is that no erythropoietic agent can be expected to be thoroughly effective as long as the basic cause of the anemia, whether it be primary or secondary, remains unknown and untreated. Moreover, when such causes have been found and removed, the natural tendency is toward blood regeneration. The situation with germanium appears quite analogous to arsenic, which likewise has been favorably recommended in the treatment of anemia and which yet has not been shown to produce the changes searched for by Minot and Sampson. On the contrary, the action of arsenic in sufficient amount, on bone marrow appears to be destructive rather than reconstructive. Anemia still remains in the vast majority of instances but a symptom of a deeper underlying pathology, and unless we can successfully eradicate the basic disease process, we but treat the symptom.

On the other hand, as long as the cause of an anemia remains unknown, attempts to stimulate the erythropoietic system would appear to be justifiable symptomatic therapy. This, provided it does not promote laxity in the search for the original pathologic process.

It may be observed that the criteria of erythropoiesis enumerated by Minot and Sampson are nonessential. This is a question which can only be definitely answered after a greater number of cases have been treated. Certainly we may conclude that there is little evidence of permanent benefit from the use of germanium dioxide in pernicious anemia. The results with secondary anemia, particularly when the cause has been found and removed, are, on the other hand, a little more hopeful.

REFERENCES

Hammett, Nowrey, and Müller: Jour. Exper. Med., 1922, xxxv, 173.
Hammett, Nowrey: Ibid., 1922, xxxv, 507.
Müller and Iszard: Am. Jour. Med. Sci., 1922, clxiii, 364.
Hammett, Müller and Nowrey: Jour. Pharm. and Exper. Therap., 1922, xix, 337.
Nowrey: Bull. Johns Hopkins Hosp., 1922, xxxiii, 340.
Müller and Iszard: Jour. Metab. Res., 1923, iii, 181.
Lenker: Penn. Med. Jour., 1922, xxvi, 86.
Kast, Croll and Schmitz: JOUR. LAB. AND CLIN. MED., 1922, vii, 643.
Minot and Sampson: Boston Med. and Surg. Jour., 1923, clxxxix, 629.

—*W. T. V.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

Medical Clinics of North America. Vol. 7. Numbers 1 and 2*

FOLLOWING past policy, the editors of the Medical Clinics have dedicated these numbers to two medical centers of the country and they contain contributions from these sources.

In the Mayo Clinic number one finds a breadth of subjects that should carry interest to all clinicians. They serve to emphasize the wide variety of medical material which finds its way to Rochester. Of chief temporal interest is the group of clinics dealing with insulin therapy. The Rochester group of insulin investigators inclines more than the others towards single daily injections, unless the dosage exceeds 30 units, and lays less emphasis on the necessity for maintaining a low blood sugar level. Although this is without doubt easier for the patient, it has not yet been demonstrated that the end results, particularly with regard to the secondary degenerative processes will be as satisfactory.

The Chicago number is equally varied in subject matter. A series of articles on various gastrointestinal disorders makes it particularly attractive to abdominal surgeons and gastroenterologists.

The great value of The Medical Clinics of North America, as an institution among current periodicals, lies in what we may term the "freshness" of the work, in that experimental work may be discussed in a clinic before it has advanced to such a stage that it may be placed in the form of a finished thesis. Also in the direct rhetoric of clinical discussion various points can be more easily emphasized than in more formal presentations. At the same time the intrinsic scientific value of such contributions is often lessened by omission of protocols, statistics, and other essential detail.

Treatment of Diabetes Mellitus†

THE third edition of Dr. Joslin's monograph covers diabetes from every angle. With characteristic modesty the author gives abundant credit to the work of Allen on undernutrition, that of Newburgh and Marsh and of Petrén in increasing the fat intake, with low protein intake; that of Shaffer and of Woodyatt in scientific balancing of the ketogenic-antiketogenic factors, and finally to Banting and his friend Best for their well-known work in

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*The Medical Clinics of North America. Mayo Clinic, Vol. 7, Number 1. Paper. Pp. 315. Illustrated. Published bi-monthly (Six numbers a year). Price per year $12.00. W. B. Saunders Company, Philadelphia, July, 1923. Chicago Number. Vol. 7. Number 2. Paper. Pp. 627. Illustrated, etc. September, 1923.

†Treatment of Diabetes Mellitus. By Elliott P. Joslin, M.D., (Harvard) M.A. (Yale) Clinical Professor of Medicine, Harvard Medical School; Consulting Physician, Boston City Hospital; Physician to New England Deaconess Hospital. Third edition, enlarged, revised and rewritten. Illustrated. Cloth. Price $8.00. Pp. 784. Lea and Febiger, Philadelphia and New York, 1923.

the development of insulin. While reading his description of the admirable work of other men, one unacquainted with the facts would be inclined to the conclusion that all of the contributions of real value had been made by others. But as one reads one realizes that Dr. Joslin's own contributions to the study and treatment of diabetes are so numerous and of such great recognized worth that they need no essayist to proclaim them. He, above all others, has pioneered in the popularization and simplification of rational diabetic therapy, and the personal experiences which he details are written in such clear and concise language that no one who attempts to really treat diabetes can afford to do so without the aid of his monograph.

Among other points he emphasizes that the diabetes of today is a new and purer diabetes, free from many of the complications which crowd and confuse the older writings. There is little alcoholism, relatively little syphilis and fewer cases are associated with tuberculosis. The incidence of diabetic complications is reduced. Gangrene, however, continues to be one of the most important complications, with more than one-fifth of all diabetics in Boston dying thereof. The author continues to look upon obesity as the greatest single predisposing cause of diabetes.

Although chapters are devoted to insulin, the use of this remedy is discussed throughout the book. In other words, the third edition is not merely a padding of the second, but is essentially a rewriting rendered necessary by our newer knowledge.

Treatment in all of its phases receives ample consideration. The usual dietary tables and schedules are included as formerly and a section is also devoted to the study of basal metabolism in diabetics.

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*Blood Chemistry Colorimetric Methods**

WITH the general employment of blood chemical studies in practice it becomes desirable that the technic of the various tests be made readily available for those attempting this work. The author has collected the methods commonly used in blood chemistry, carefully eliminating unnecessary variations and tests infrequently used, and after describing each procedure in detail, has discussed the clinical significance of results. A brief chapter describing certain diets would gain in value were it more comprehensive.

Although the book is written for the general practitioner it is a question whether many general practitioners have the time or the experience to correctly perform the tests described, and it is indeed a question whether we are not fostering inaccurate work in making the various methods appear too simple. On the other hand, all general practitioners who have occasion to have this work done and receive reports thereon should know what procedures have been necessary to arrive at the conclusions. For this reason and for the distinct help to be obtained from the sections dealing with the clinical interpretation of results, this volume should be of use to the general practitioner.

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*Blood Chemistry Colorimetric Methods. By Willard J. Stone, M.D., Pasadena, Cal. Attending Physician, Los Angeles General Hospital. Introduction by George Dock, M.D., Pasadena, Cal. Cloth. Price $2.25. Pp. 75. Paul B. Hoeber, Inc., New York, 1923.

*The Medical Department in the World War**

THE first volume of the Surgeon General's report on the work of the medical department of the United States Army in the World War deals with the surgeon general's office, its organization, personnel and accomplishments. A brief history of the development of medical work in connection with armies from ancient times is unusually interesting. In the 1400 pages of the report there is much statistical detail, and a considerable portion of the book is devoted to medical department promulgations which have a distinct reference value but do not make easy reading. This of course is a necessary feature of all such reports and will be of inestimable value in the next war. In fact, one is struck not only by the tremendous advances that were made by the medical department during the war but also by the plans that are being perfected for greater future efficiency based upon the experience gained.

The evolution of the greater surgeon general's office during the war is interesting reading as a historical document. The development and work of the various departments is treated in an entirely impersonal manner with little or no mention of the accomplishments of individual members. One can readily understand how it is necessary to avoid such discussions, for although they alone would lend human interest to the report, and would people the otherwise empty surgeon general's office with active working souls, they would at the same time work an unfairness in emphasizing the contributions of some, while underrating the equally valuable work of others. Thus, we are interested to know that "on April 14, 1916, a group of eminent surgeons met in the Union League Club in Chicago and organized a Committee on Medical Preparedness," that in August, 1916, a medical representative was placed on the council of executive information; and again that "the organization of the advisory council provided that each member of the commission gather about himself for the most effective coordination of the activities he represented, a committee or board of representatives of the Government departments and of eminent civilian members." And it is interesting to know that these organizations worked actively throughout the year preceding our entry into the war. But how much more interesting would it be to read of what these various organizations preparing for war actually did accomplish, how it was discovered that surgical needles were imported nearly exclusively from England and what steps were taken to provide an adequate supply in this country, how inventory was made of all available medical supplies, and, as in the case of digitalis, it was found necessary to utilize an hitherto unused home-grown product which incidentally was then found to be more potent than the imported drug. All of this was accomplished quietly and without ostentation long before war was declared. It is regrettable that in a formal government document discussions of "human interest" must be eliminated.

Volume One of the Surgeon General's report will become an invaluable reference work.

*The Medical Department of the United States Army in the World War. Vol. I. The Surgeon General's Office. Prepared under the direction of Maj. Gen. M. W. Ireland, M.D., Surgeon General of the Army. By Col. Charles Lynch, M.C., Lieut. Col. Frank W. Weed, M.C., Loy McAlfee, A.M., M.D. Cloth. Pp. 1389. Government Printing Office, Washington, D. C., 1923.

NEWS AND NOTES

Philadelphia Academy of Surgery

The Samuel D. Gross Prize

Fifteen Hundred Dollars

Essays will be received in competition for the prize until January 1, 1925.

The conditions annexed by the testator are that the prize "shall be awarded every five years to the writer of the best original essay, not exceeding one hundred and fifty printed pages, octavo, in length, illustrative of some subject in Surgical Pathology or Surgical Practice, founded upon original investigations, the candidates for the prize to be American citizens."

It is expressly stipulated that the competitor who receives the prize shall publish his essay in book form, and that he shall deposit one copy of the work in the Samuel D. Gross Library of the Philadelphia Academy of Surgery, and that on the title page it shall be stated that to the essay was awarded the Samuel D. Gross Prize of the Philadelphia Academy of Surgery.

The essays, which must be written by a single author in the English language, should be sent to the "Trustees of the Samuel D. Gross Prize of the Philadelphia Academy of Surgery, care of the College of Physicians, 19 S. 22nd St., Philadelphia," on or before January 1, 1925.

Each essay must be typewritten, distinguished by a motto, and accompanied by a sealed envelope bearing the same motto, containing the name and address of the writer. No envelope will be opened except that which accompanies the successful essay.

The Committee will return the unsuccessful essays if reclaimed by their respective writers, or their agents, within one year.

The Committee reserves the right to make no award if the essays submitted are not considered worthy of the prize.

WILLIAM J. TAYLOR, M.D.,
JOHN H. JOPSON, M.D.,
EDWARD B. HODGE, M.D.,
Trustees.

Philadelphia, March 15, 1924.